

**EMMA ID:** 08080

**Gene:** *Becn1*

**Common name:** *EPD0168\_1\_H11*

**Allele:** *Becn1*<sup>tm1b(KOMP)Wtsi</sup>

## 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:1891828/tm1b\(KOMP\)Wtsi](http://www.mousephenotype.org/data/alleles/MGI:1891828/tm1b(KOMP)Wtsi)

## 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   | Becn1 5'arm    | LAR3           | 415                     |
| Wildtype | Becn1 5'arm    | Becn1 3'arm    | 624                     |

### Primer sequences

| Primer Name | Sequence 5' --> 3'     |
|-------------|------------------------|
| Becn1 5'arm | gtcatatgtacacaggctgtg  |
| Becn1 3'arm | ccaactcctgagaactgtttg  |
| LAR3        | CAACGGGTTCTTCTGTTAGTCC |

### PCR setup (Qiagen, Hot Start Plus)

| Component                     | Volume ( $\mu$ 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 <sub>2</sub> (25 mM)     | 1,5                  | 1,5         |
| Primer 1 (10 pmol/ $\mu$ l)   | 1                    | 0,4         |
| Primer 2 (10 pmol/ $\mu$ l)   | 1                    | 0,4         |
| Taq Polymerase (5 U/ $\mu$ l) | 0,3                  | 0,06        |
| H <sub>2</sub> O*             | 13,7                 |             |
| Final volume                  | 25                   |             |

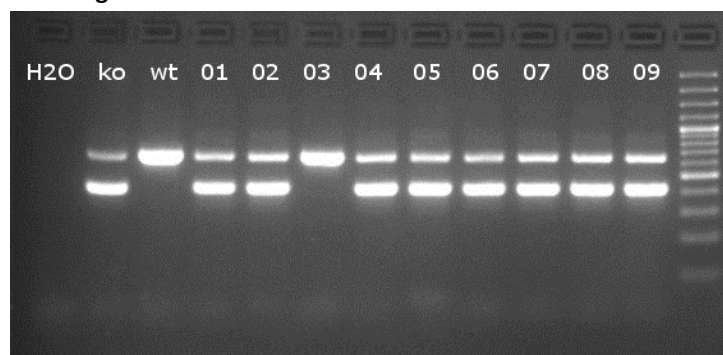
\* The amount of H<sub>2</sub>O is adjusted with the number of primer.

### Amplification conditions

| PCR Settings                                       | Temperature ( $^{\circ}$ C) | Time   | # of cycles |
|--|-----------------------------|--------|-------------|
| 1 Denaturation<br>(Melting)                        | 95 $^{\circ}$ C             | 5 min  | 1           |
| 2 Amplification<br>(Melting, Annealing,<br>Polym.) | 94 $^{\circ}$ C             | 30 sec | 39          |
|  | 65 $^{\circ}$ 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



Separated by gel electrophoresis on a 2% agarose gel.

Work as Triplex.

## 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 <sub>2</sub> (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 <sub>2</sub> 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<br>Promotor-driven | tm1b_forw      | Floxed LR      | 380 bp<br>others | tm1b, Promotor-driven<br>tm1a or partially conversion |
| Flox<br>Promotorless    | Floxed PNF     | Floxed LR      | 128 bp<br>~ 1 kb | tm1b, Promotorless<br>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 <sub>2</sub> 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.