

**EMMA ID:** 12826  
**Gene:** *ErbB2*  
**Common name:** *HEPD0668\_3\_H11*  
**Allele:** *ErbB2*<sup>tm1b(EUCOMM)Hmgu</sup>

## 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/genes/MGI:95410>

## 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   | ErbB2 5'arm    | LAR3           | 530                     |
| Wildtype | ErbB2 5'arm    | ErbB2 3'arm    | 425                     |

### Primer sequences

| Primer Name | Sequence 5' --> 3'    |
|-------------|-----------------------|
| ErbB2 5'arm | aagagctctcccatgatgacc |
| ErbB2 3'arm | acaggcctagagtagaatgc  |
| LAR3        | CAACGGGTTCTTCTGTAGTCC |

### 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 <sub>2</sub> (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 <sub>2</sub> O*         | 13,7           |             |
| Final volume              | 25             |             |

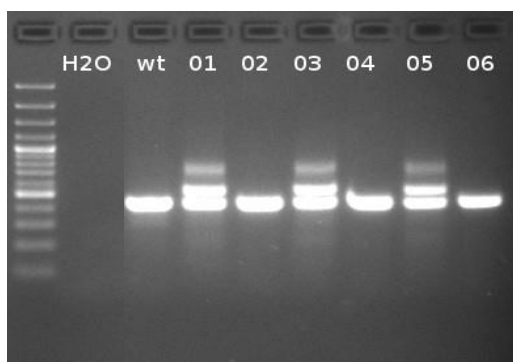
\* The amount of H<sub>2</sub>O is adjusted with the number of primer.

### Amplification conditions

| PCR Settings                                       | Temperature (°C)   | Time   | # of cycles |
|--|--------------------|--------|-------------|
| 1 Denaturation<br>(Melting)                        | 95°C               | 5 min  | 1           |
| 2 Amplification<br>(Melting, Annealing,<br>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           |

use 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.

## 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                                    | 4°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         | tm1b, Promotor-driven        |
|                         |                |                | others         | tm1a or partially conversion |
| Floxed<br>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 <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            | 4°C              | hold   |

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