

**EMMA ID:** 10129

**Gene:** *Ech1*

**Common name:** *EPD0370\_2\_B11*

**Allele:** *Ech1*<sup>tm1b(EUCOMM)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:1858208/tm1b\(EUCOMM\)Wtsi](http://www.mousephenotype.org/data/alleles/MGI:1858208/tm1b(EUCOMM)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	Ech1 5' arm neu	LAR3	458
Wildtype	Ech1 5' arm neu	Ech1 3' arm neu	673

### Primer sequences

Primer Name	Sequence 5' --> 3'
Ech1 5' arm neu	gctggtcctaagaatgccagt
Ech1 3' arm neu	gttgagaaggagatgagaggt
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	

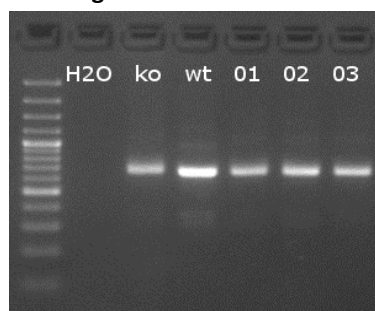
\* The amount of H<sub>2</sub>O is adjusted with the number of primer.

### Amplification conditions

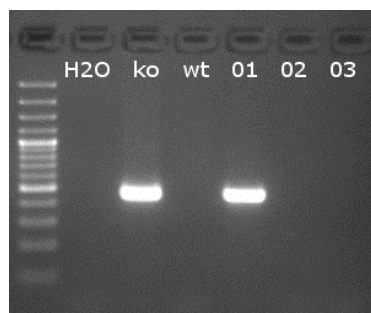
PCR Settings	Temperature ( $^{\circ}$ C)	Time	# of cycles
1 Denaturation (Melting)	95 $^{\circ}$ C	5 min	1
2 Amplification (Melting, Annealing, Polym.)	94 $^{\circ}$ C	30 sec	39
	65 $^{\circ}$ C	45 sec	
	72 $^{\circ}$ C	45 sec	
3 Polymerisation	72 $^{\circ}$ C	10 min	1
4 Cooling	12 $^{\circ}$ C	hold	1

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

### Gel Image



**WT-PCR**



**Mutant-PCR**

Separated by gel electrophoresis on a 2% agarose gel.

WT-PCR doesn't work very well. High quality of DNA samples is suggested.

## 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 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 <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.**