EMMA ID: *08100*
Gene: *Nxn*
Common name: *EPD0045_1_B11*
Allele: *Nxn* $^{tm1b(EUCOMM)Wtsi}$

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:109331/tm1b%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	Nxn neu2 5'arm	LAR3	543
Wildtype	Nxn neu2 5'arm	Nxn neu2 3'arm	477

Primer sequences

Primer Name	Sequence 5' --> 3'
Nxn neu2 5'arm	TGTAGCAAGCCCAACATTTTC
Nxn neu2 3'arm	GACCTTAACCAGCTGACTCAG
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	

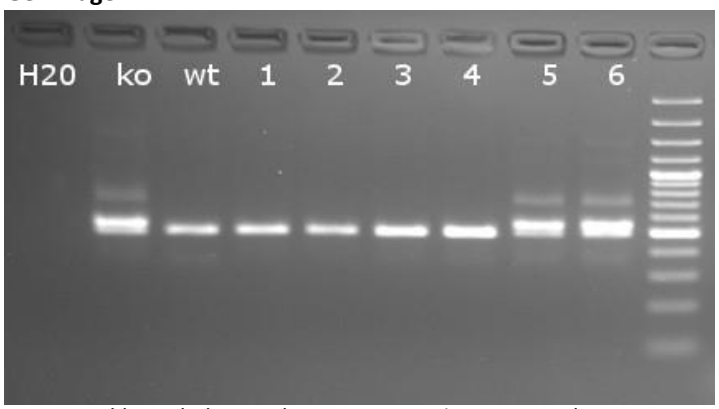
*** The amount of H_2O is adjusted with the number of primer.**

Amplification conditions

PCR Settings	Temperature (°C)	Time	# of cyles
1 Denaturation (Melting)	95°C	5 min	1
2 Amplification (Melting, Annealing, Polym.)	94°C 65°C 72°C	30 sec 45 sec 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



Separated by gel electrophoresis on a 2% agarose gel.
homozygous with wt band

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 58°C 72°C	30 sec 45 sec 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.

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 togehter 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_2O	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.