EMMA ID: *09249*
Gene: *Smc6*
Common name: *HEPD0531_1_D05*
Allele: *Smc6* $^{tm1b(EUCOMM)Hmgu}$

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:1914491/tm1b%2528EUCOMM%2529Hmgu?

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	Smc6_Ef	KR 3277	206
Wildtype	-	-	-

Primer sequences

Primer Name	Sequence 5' --> 3'
Smc6_Ef	GCTGATTCTGAGGAGGCAG
KR 3277	CTCCTACATAGTTGGCAGTGTTTGGG

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	

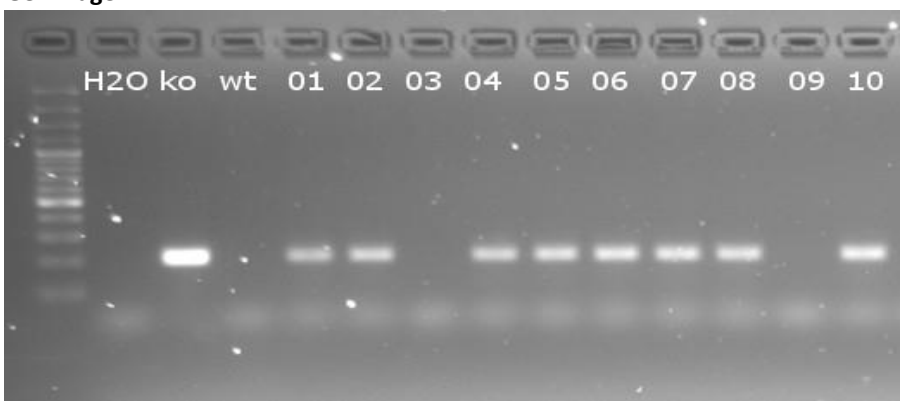
*** The amount of H_2O is adjusted with the number of primer.**

Amplification conditions

PCR Settings	Temperature (°C)	Time	# of cyles
1 Denaturation (Melting)	95°C	5 min	1
2 Amplification (Melting, Annealing, Polym.)	94°C 62°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.
only ko-pcr

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.