

EMMA ID: 06276

Gene: *Rab35*

Common name: *HEPD0537_1_F06*

Allele: *Rab35*^{tm1a(EUCOMM)Hmgu}

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:1924657/tm1a\(EUCOMM\)Hmgu](http://www.mousephenotype.org/data/alleles/MGI:1924657/tm1a(EUCOMM)Hmgu)

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	Rab35 5'arm	LAR3	423
Wildtype	Rab35 5'arm	Rab35 3'arm	370

Primer sequences

Primer Name	Sequence 5' --> 3'
Rab35 5'arm	ctgctaagcgttctactacct
Rab35 3'arm	tgaggacagccttcaaagctg
LAR3	CAACGGGTTCTTCTGTAGTCC

PCR setup (Qiagen, Hot Start Plus)

Component	Volume (μ 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 ₂ (25 mM)	1,5	1,5
Primer 1 (10 pmol/ μ l)	1	0,4
Primer 2 (10 pmol/ μ l)	1	0,4
Taq Polymerase (5 U/ μ l)	0,3	0,06
H ₂ O*	13,7	
Final volume	25	

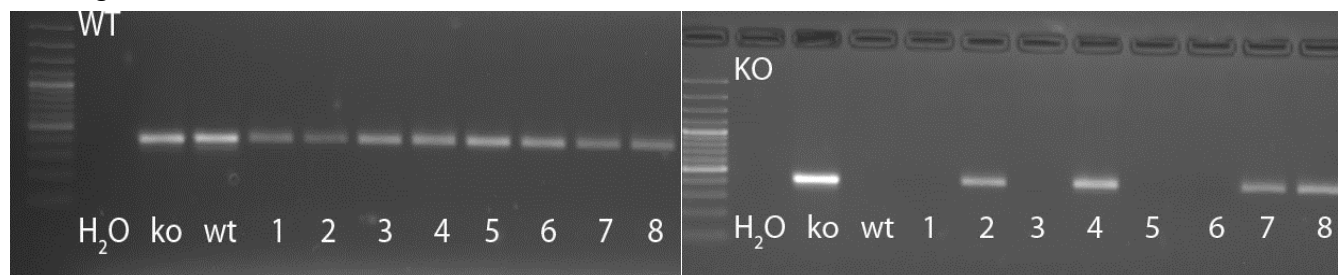
* The amount of H₂O is adjusted with the number of primer.

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.

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 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 ₂ 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.