

Gene: Rhot2

Colony prefix: MCSF

ESC clone ID: EPD0389_5_A05

Allele: *Rhot2*^{tm1(KOMP)Wtsi}

Allele type: Deletion

Allele information:

Further information about the allele can be found on the 'International Mouse Phenotyping Consortium' (IMPC) web site at http://www.mousephenotype.org/martsearch_ikmc_project/martsearch/ikmc_project/33961
Details on how to determine the floxed exon can be found at <http://www.i-dcc.org/kb/entry/21/>

Mouse QC information



Southern Blot	na	TV Backbone Assay	pass	5' LR-PCR	na
Loss of WT Allele (LOA) qPCR	pass	Homozygous Loss of WT Allele (LOA) SR-PCR	pass	Neo Count (qPCR)	pass
LacZ SR-PCR	pass	5' Cassette Integrity	pass	Neo SR-PCR	na
Mutant Specific SR-PCR	pass	LoxP Confirmation	na	3' LR-PCR	na
Genotyping Comment					

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Southern blot confirmation:

Southern blots are not routinely performed at the Sanger Institute due to throughput constraints. A southern blot experiment design tool can be found on the IMPC web site at http://www.mousephenotype.org/martsearch_ikmc_project/martsearch/ikmc_project/33961

Links to information and frequently asked questions about the EUCOMM/KOMP alleles and MGP projects

General targeting strategies:

http://www.mousephenotype.org/martsearch_ikmc_project/about/targeting-strategies

MGP mouse phenotype data:

<http://www.sanger.ac.uk/mouseportal/>

IKMC allele types:

<http://www.i-dcc.org/kb/entry/89/>

MGP mouse quality control tests :

<http://www.i-dcc.org/kb/25/>

Allele conversion guide - genotyping tm1b, tm1c and tm1d mice:

<http://www.i-dcc.org/kb/entry/105/>

How the "critical" exon is decided:

<http://www.i-dcc.org/kb/entry/102/>

Genotyping Information

Genotyping by end-point PCR

These mice may be genotyped through a combination of separate PCR reactions that detect the cassette, the gene-specific wild type allele, and a mutant allele-specific short range PCR. Interpretation of the consolidated results produces the genotype of the mice.

For example: cassette positive, mutant positive, wild type positive = heterozygous.

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PCRs primer pairs and expected size bands

Assay Type	Assay	Forward Primer	Reverse Primer	Expected Size Band (bp)
Standard PCR	Cassette	LacZ_2_small_F	LacZ_2_small_R	108
Standard PCR	Wildtype	Rhot2_WT_F3	Rhot2_WT_R3	248
Standard PCR	Mutant	Rhot2_mut_F4	CAS_R1_Term	230

Primer sequences

Primer Name	Primer Sequence (5' > 3')
CAS_R1_Term	TCGTGGTATCGTTATGCGCC
LacZ_2_small_F	ATCACGACGCGCTGTATC
LacZ_2_small_R	ACATCGGGCAAATAATATCG
Rhot2_WT_F3	GGCGTTAGCTCCAGTGAGTC
Rhot2_WT_R3	AGGATGTGAAGAGGGTGGTG
Rhot2_mut_F4	GGGTTGAAGGAGAGGGTTG

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Reaction setup

Reagent	µl
DNA (~50-100 ng)	1
10x Buffer	2
MgCl ₂ (50 mM)	0.6
Platinum Taq (Invitrogen)	0.2
dNTPs (100 mM)	0.2
Primer 1 (10 µM)	0.4
Primer 2 (10 µM)	0.4
ddH ₂ O	15.2
Total	20

Amplification conditions

Step	Conditions	Time
1	94°C	5 min
2	94°C	30 sec
3	58°C	30 sec
4	72°C	45 sec
5	Go to '2' + 34 cycles	-
6	72°C	5 min
7	12°C	forever

Relevant publications

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- Bradley, A., Anastassiadis, K., Ayadi, A., Battey, J.F., Bell, C., Birling, M.-C., Bottomley, J., Brown, S.D., Bürger, A., Bult, C.J., et al. (2012). The mammalian gene function resource: the international knockout mouse consortium. *Mamm Genome* 23, 580–586.
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