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Gene: Rhou

Colony prefix: MBZM

ESC clone ID: EPD0107 4 E05

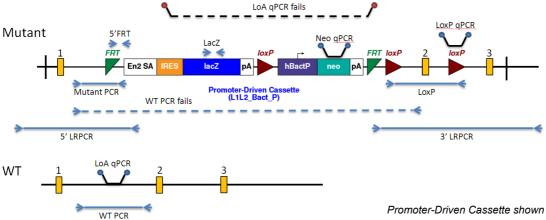
Allele: Rhoutm1a(KOMP)Wtsi

Allele type: Knockout First, Reporter-tagged insertion with conditional potential

## 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/24761 Details on how to determine the floxed exon can be found at http://www.i-dcc.org/kb/entry/21/

## Mouse QC information



Promoter-Driven Cassette shown for illustrative purposes

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	pass	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 <a href="http://www.mousephenotype.org/martsearch\_ikmc\_project/24761">http://www.mousephenotype.org/martsearch\_ikmc\_project/24761</a>

# 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	Mutant	Rhou_45894_F	CAS_R1_Term	265
Standard PCR	Wildtype	Rhou_45894_F	Rhou_45894_R	392

# **Primer sequences**

Primer Name	Primer Sequence (5' > 3')	
CAS_R1_Term	TCGTGGTATCGTTATGCGCC	
LacZ_2_small_F	ATCACGACGCGCTGTATC	
LacZ_2_small_R	ACATCGGGCAAATAATATCG	
Rhou_45894_F	AGCAAGTTCTCAAGGGTGGG	
Rhou_45894_R	ACAGATTTTCCCCCAGGGTC	

# **Reaction setup**

Reagent	μΙ
DNA (~50-100 ng)	1
10x Buffer	2
MgCl2 (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
ddH20	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

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## Genotyping using universal copy number qPCR assays designed to the selection cassette

The cassette qPCR assays use a hydrolysis probe assay (eg Applied Biosystems TaqMan technology) to determine genotype via the copy number of the selection cassette in a sample. Homozygotes will possess two copies, heterozygotes one copy and wild type mice will show no amplification when compared to known homozygote controls.

These FAM®-labeled assays are multiplexed with a VIC® labeled endogenous control assay (for example TaqMan® Copy Number Reference Assay, Mouse, Tfrc; Applied Biosystems part #4458366).

Please note that these assays are not gene-specific – other information should be used in conjunction with the universal cassette assays (for example the mutant-specific srPCR) when confirming the gene identity.

Primer type	Assay Name	Forward Primer Seq.	Reverse Primer Seq.	Probe Primer Seq.
Cassette	Neo	GGTGGAGAGGCTATTCGGC	GAACACGGCGGCATCAG	TGGGCACAACAGACAATCGGCT G

Reactions are performed in a 10µl volume using an Applied Biosystems 7900HT Fast Real-Time PCR System or Applied Biosystems Viia7 with DNA prepared using the Sample-to-SNP<sup>TM</sup> kit (Applied Biosystems) from mouse ear biopsies. GTXpress<sup>TM</sup> buffer is also used (Applied Biosystems).

Reagent	μΙ
2x GTXpress <sup>™</sup> buffer	5
20x target assay	0.5
ddH2O	3
Tfrc endogenous 20x assay	0.5
DNA	1

#### **Amplification conditions**

Step	Conditions	Time
1	95°C	20 sec
2	95°C	10 sec
3	60°C	30 sec
4	Go to '2' + 34 cycles	-

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## Genotyping by loss of WT allele qPCR Assay (gene-specific assay)

The wild type loss of allele (LoA) qPCR assay uses a hydrolysis probe assay (for example Applied Biosystems TaqMan® technology) to determine the copy number of the wild type allele in a sample. Homozygotes will show no amplification, heterozygotes one copy and wild type mice will show two copies when compared to a wild type control.

The number of copies of the Rhou allele can be detected using a FAM-labelled custom qPCR TaqMan® assay. These are multiplexed with a VIC® labelled endogenous control assay (for example TaqMan® Copy Number Reference Assay, Mouse, Tfrc; Applied Biosystems part #4458366). Reference DNA controls of known genotypes should also be included to facilitate correct analysis.

## Primers for LoA gPCR assay

Primer type	Assay Name	Forward Primer Seq.	Reverse Primer Seq.	Probe Primer Seq.
LoA	Rhou_WT	CACCGCTCACTAGCATAAACATT T	GTAAAGACTCGGAGGTTTCAGCT TA	CCTTCTGATAGCATTTCTG

### Reaction setup

Reaction setup and amplification conditions are the same as those used for the neo cassette qPCR assay.

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## Relevant publications

Ryder, E., Doe, B., Gleeson, D., Houghton, R., Dalvi, P., Grau, E., Ramirez-Solis, R. (2013). Rapid conversion of EUCOMM/KOMP-CSD alleles in mouse embryos using a cell-permeable Cre recombinase. Transgenic research. 23(1), 177–185.

Ryder, E., Gleeson, D., Sethi, D., Vyas, S., Miklejewska, E., Dalvi, P., Habib, B., Cook, R., Hardy, M., Jhaveri, K., et al. (2013). Molecular Characterization of Mutant Mouse Strains Generated from the EUCOMM/KOMP-CSD ES Cell Resource. Mamm. Genome. 24, 286–294.

White, J.K., Gerdin, A.-K., Karp, N.A., Ryder, E., Buljan, M., Bussell, J.N., Salisbury, J., Clare, S., Ingham, N.J., Podrini, C., et al. (2013). Genome-wide Generation and Systematic Phenotyping of Knockout Mice Reveals New Roles for Many Genes. Cell 154, 452–464.

Ryder, E., Wong, K., Gleeson, D., Keane, T.M., Sethi, D., Vyas, S., Wardle-Jones, H., Bussell, J.N., Houghton, R., Salisbury, J., et al. (2013). Genomic analysis of a novel spontaneous albino C57BL/6N mouse strain. Genesis 51, 523–528.

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.

Birling, M.-C., Dierich, A., Jacquot, S., Hérault, Y., and Pavlovic, G. (2011). Highly-efficient, fluorescent, locus directed Cre and flpo deleter mice on a pure C57BL/6N genetic background. Genesis.

Skarnes, W.C., Rosen, B., West, A.P., Koutsourakis, M., Bushell, W., Iyer, V., Mujica, A.O., Thomas, M., Harrow, J., Cox, T., et al. (2011). A conditional knockout resource for the genome-wide study of mouse gene function. Nature 474, 337–342.

Pettitt, S.J., Liang, Q., Rairdan, X.Y., Moran, J.L., Prosser, H.M., Beier, D.R., Lloyd, K.C., Bradley, A., and Skarnes, W.C. (2009). Agouti C57BL/6N embryonic stem cells for mouse genetic resources. Nat Methods 6, 493–495.

Liang, Q., Conte, N., Skarnes, W.C., and Bradley, A. (2008). Extensive genomic copy number variation in embryonic stem cells. Proc Natl Acad Sci U S A 105, 17453–17456.

Farley, F.W., Soriano, P., Steffen, L.S., and Dymecki, S.M. (2000). Widespread recombinase expression using FLPeR (flipper) mice. Genesis 28, 106–110.

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