

Gene: Grin1

Colony prefix: MDCY

ESC clone ID: EPD0469_5_C11

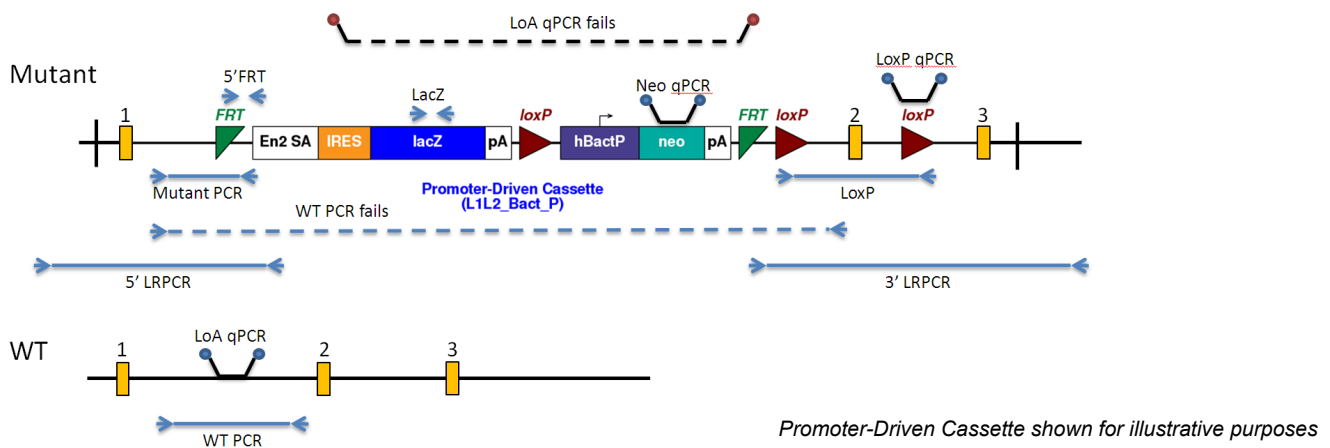
Allele: *Grin1^{tm1a(EUCOMM)Wtsi}*

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/36828
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	na	5' LR-PCR	na
Loss of WT Allele (LOA) qPCR	na	Homozygous Loss of WT Allele (LOA) SR-PCR	na	Neo Count (qPCR)	na
LacZ SR-PCR	na	5' Cassette Integrity	na	Neo SR-PCR	na
Mutant Specific SR-PCR	na	LoxP Confirmation	na	3' LR-PCR	na
Genotyping Comment					

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Southern blot confirmation:

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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	Wildtype	Grin1_26373_F	Grin1_26373_R	288
Standard PCR	Mutant	Grin1_26373_F	CAS_R1_Term	218
Standard PCR	Cassette	LacZ_2_small_F	LacZ_2_small_R	108

Primer sequences

Primer Name	Primer Sequence (5' > 3')
CAS_R1_Term	TCGTGGTATCGTTATGCGCC
Grin1_26373_F	CGCATCCTCAAGACAAGCAC
Grin1_26373_R	CATGCAAGCTGAAATGGACG
LacZ_2_small_F	ATCACGACGCGCTGTATC
LacZ_2_small_R	ACATCGGGCAAATAATATCG

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 M)	0.4
Primer 2 (10 M)	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

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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 Grin1 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, Tfr; Applied Biosystems part #4458366). Reference DNA controls of known genotypes should also be included to facilitate correct analysis.

Primers for LoA qPCR assay

Primer type	Assay Name	Forward Primer Seq.	Reverse Primer Seq.	Probe Primer Seq.
LoA	Grin1_WT	CGTGCAGCGAACAGGAAATAGA	TCAGCCCCATCTCTGTTCTA	TTGCCTTCCAAAACITTT

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

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- 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.
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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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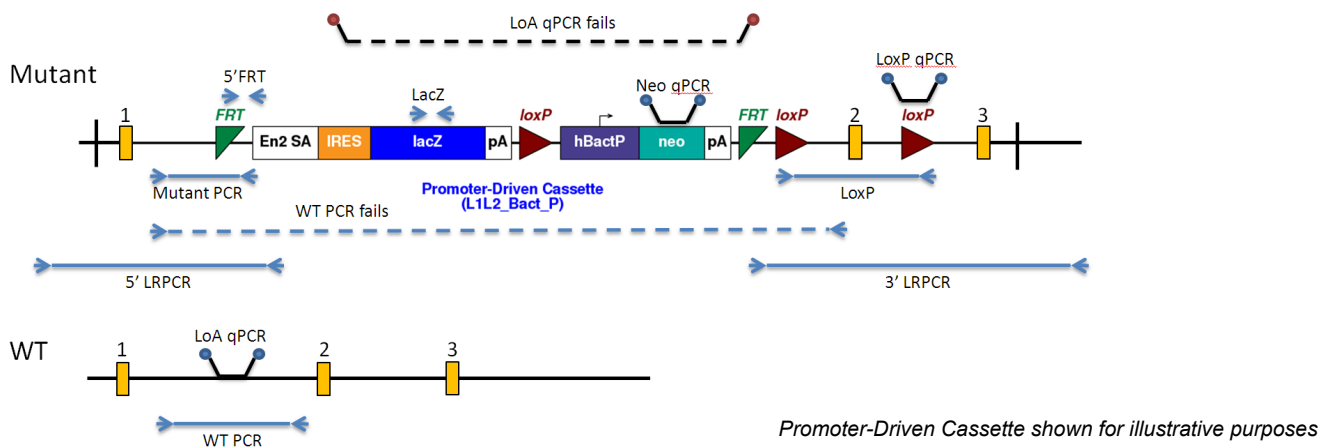
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LacZ SR-PCR	pass	5' Cassette Integrity	pass	Neo SR-PCR	na
Mutant Specific SR-PCR	pass	LoxP Confirmation	pass	3' LR-PCR	na
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Primer sequences

Primer Name	Primer Sequence (5' > 3')
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Reaction setup

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DNA (~50-100 ng)	1
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Step	Conditions	Time
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Primers for LoA qPCR assay

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