Wellcome Sanger Institute
Wellcome Genome Campus
Hinxton Cambridge CB10 1SA, U.K.

MGPgenotyping@sanger.ac.uk
www.sanger.ac.uk

Gene: 2310057J18Rik

Colony prefix: *MCSX*

ESC clone ID: *EPD0101_4_E03*

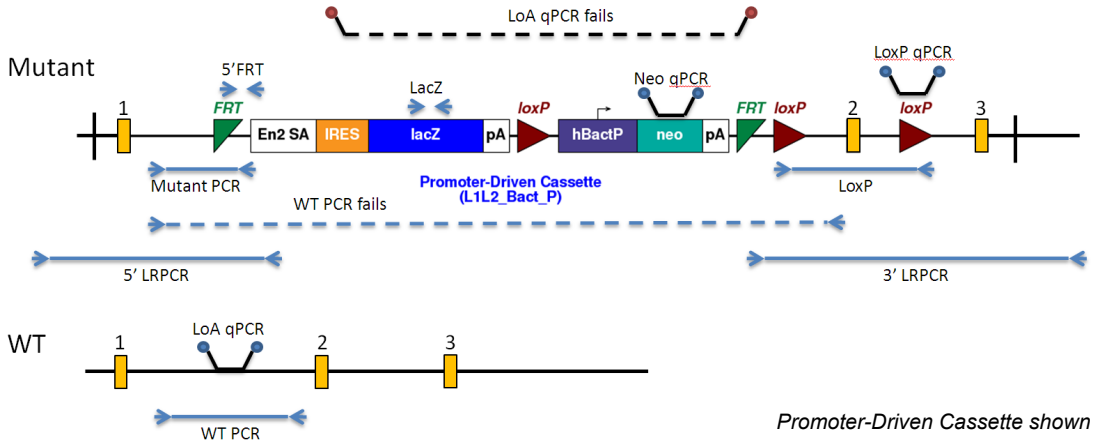
Allele: *2310057J18Rik$^{tm1a(KOMP)Wtsi}$*

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/28704
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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IKMC allele types:
http://www.i-dcc.org/kb/entry/89/

MGP mouse quality control tests :
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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	2310057J18Rik_46093_F	CAS_R1_Term	114
Standard PCR	Wildtype	2310057J18Rik_46093_F	2310057J18Rik_46093_R	475

Primer sequences

Primer Name	Primer Sequence (5' > 3')
2310057J18Rik_46093_F	TTGATCCTCTACAATGGGAAAGC
2310057J18Rik_46093_R	TTCACAGATCCCGAATCACAG
CAS_R1_Term	TCGTGGTATCGTTATGCGCC
LacZ_2_small_F	ATCACGACGCGCTGTATC
LacZ_2_small_R	ACATCGGGCAAATAATATCG

Reaction setup

Reagent	µl
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	TGGGCACAACAGACAATCGGCTG

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^{TM} kit (Applied Biosystems) from mouse ear biopsies. GTXpress^{TM} buffer is also used (Applied Biosystems).

Reagent	µl
2x GTXpress^{TM} 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
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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 2310057J18Rik 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 qPCR assay

Primer type	Assay Name	Forward Primer Seq.	Reverse Primer Seq.	Probe Primer Seq.
LoA	2310057J18Rik_WT	TTGGTAAAGGCATACTTTATATTTTACAACTTAGACT	GGGTACCAGTCAAAAAGTTTTGCTTTAG	TTGGTGATATTCTGTATAAAAAT

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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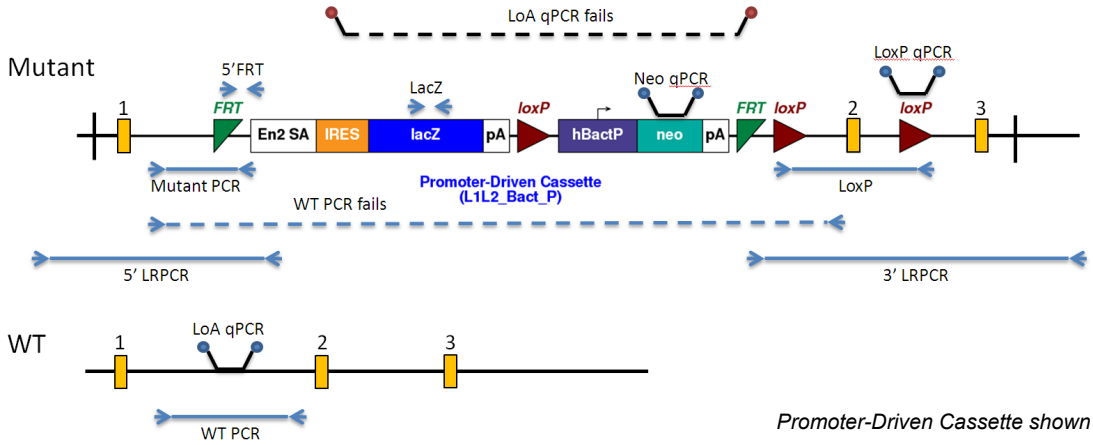

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LacZ SR-PCR	na	**5' Cassette Integrity**	na	**Neo SR-PCR**	na
Mutant Specific SR-PCR	na	**LoxP Confirmation**	na	**3' LR-PCR**	na
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