

Gene: 3110001I22Rik

Colony prefix: MCSH

ESC clone ID: EPD0113_2_B01

Allele: 3110001I22Rik^{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/28411
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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Links to information and frequently asked questions about the EUCOMM/KOMP alleles and MGP projects

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MGP mouse phenotype data:

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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.

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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	3110001I22Rik_46622_F	CAS_R1_Term	141
Standard PCR	Wildtype	3110001I22Rik_46622_F	3110001I22Rik_46622_R	402

Primer sequences

Primer Name	Primer Sequence (5' > 3')
3110001I22Rik_46622_F	AGGATTGCAGCATCAGGAGG
3110001I22Rik_46622_R	CCTCCCAGGTTGGTCTTGTC
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
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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The number of copies of the 3110001122Rik 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	3110001122Rik_W T	GCTAGGTCACGTGTATGATTCAA GA	ACTCTGTCTCAAAAAACAAAAAC AATCGA	ACAAATGAGCAAATTAC

Reaction setup

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

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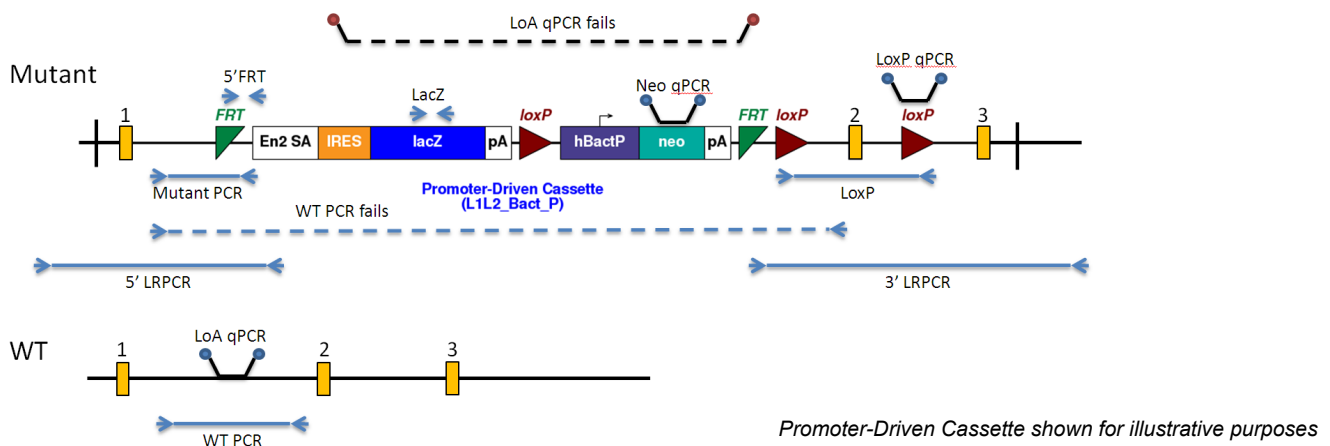
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LacZ SR-PCR	pass	5' Cassette Integrity	pass	Neo SR-PCR	na
Mutant Specific SR-PCR	pass	LoxP Confirmation	pass	3' LR-PCR	na
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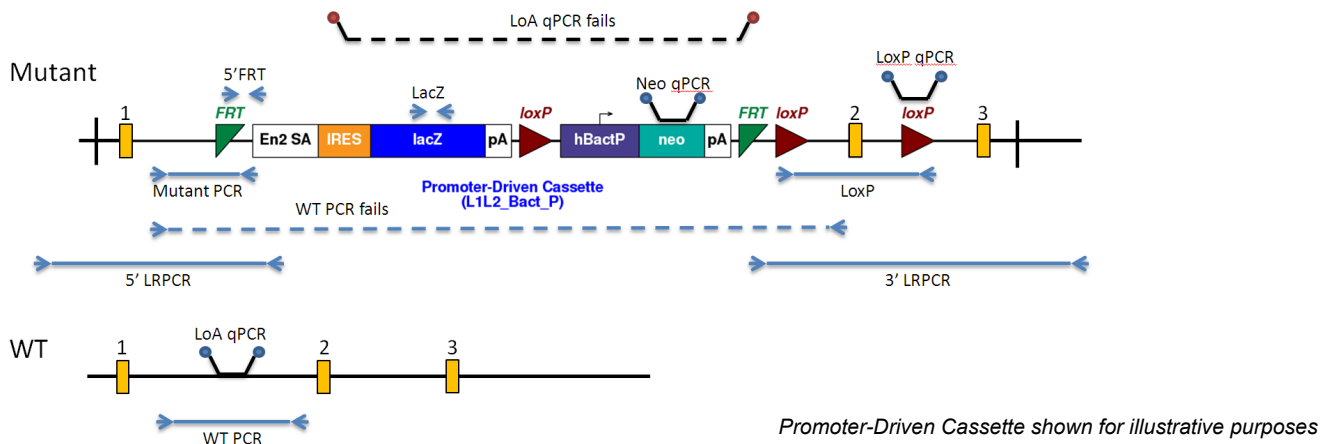
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LoA	3110001I22Rik_W T	GCTAGGTCACGTGTATGATTCAA GA	ACTCTGTCTCAAAAAACAAAAAC AATCGA	ACAAATGAGCAAATTAC

Reaction setup

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

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