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Gene: Sec24a

Colony prefix: MBQR

ESC clone ID: EPD0099_2_E04

Allele: Sec24atm1a(KOMP)Wtsi

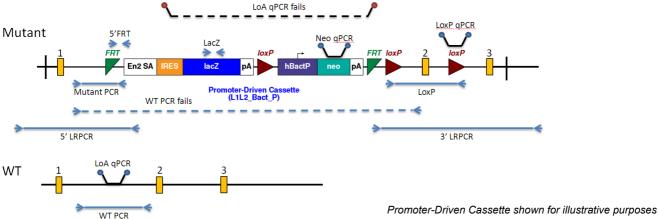
Allele type: Knockout First, Reporter-tagged insertion with conditional potential

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/24915

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

Mouse QC information



WITCK					
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	na	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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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

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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	Sec24a_44888_F	CAS_R1_Term	154
Standard PCR	Wildtype	Sec24a_44888_F	Sec24a_44888_R	335

Primer sequences

Primer Name	Primer Sequence (5' > 3')	
CAS_R1_Term	TCGTGGTATCGTTATGCGCC	
LacZ_2_small_F	ATCACGACGCGCTGTATC	
LacZ_2_small_R	ACATCGGGCAAATAATATCG	
Sec24a_44888_F	GGAGAACCACTTGACCCAGAAG	
Sec24a_44888_R	CCTTTAATCCCAGCACCCAG	

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 M)	0.4
Primer 2 (10 M)	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	-
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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 Sec24a 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

	<u> </u>			
Primer type	Assay Name	Forward Primer Seq.	Reverse Primer Seq.	Probe Primer Seq.
	•	·	·	•
LoA	SEC24A WT	TCCTGGTACAATAGCCTGGACTA	AGGTATTTGTTTGAGGATTGAGT	CTGCTAATATCAAGGTAAGATTAA
	0202	AT		
		Al	CATCAC	

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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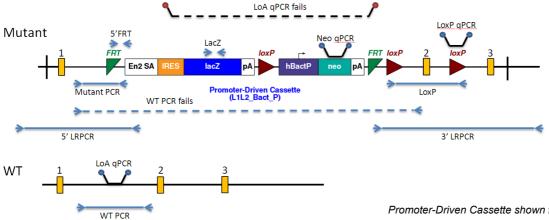
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Promoter-Driven Cassette shown for illustrative purposes

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
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Primer sequences

Primer Name	Primer Sequence (5' > 3')	
CAS_R1_Term	TCGTGGTATCGTTATGCGCC	
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Reaction setup

Reagent	μΙ
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10x Buffer	2
MgCl2 (50 mM)	0.6
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Step	Conditions	Time
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Primers for LoA qPCR assay

Primer type	Assay Name	Forward Primer Seq.	Reverse Primer Seq.	Probe Primer Seq.
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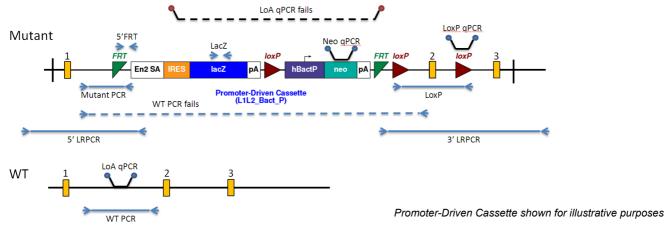
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