

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

Gene: Capzb

Colony prefix: MBLU

ESC clone ID: *EPD0105_5_A09* **Allele**: *Capzb*^{tm1a(EUCOMM)Wtsi}

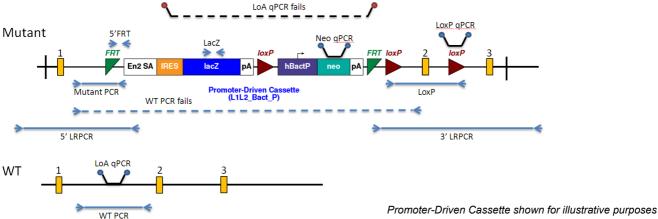
Allele type: Knockout First, Reporter-tagged insertion with conditional potential

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/38409

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 TV Backbone Assay 5' LR-PCR na Loss of WT Allele Homozygous Loss of WT Neo Count (qPCR) na na na Allele (LOA) SR-PCR (LOA) qPCR LacZ SR-PCR 5' Cassette Integrity Neo SR-PCR na na na Mutant Specific SR-LoxP Confirmation 3' LR-PCR na **PCR 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 http://www.mousephenotype.org/martsearch_ikmc_project/38409

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	Wildtype	Capzb_47322_F	Capzb_47322_R	419
Standard PCR	Mutant	Capzb_47322_F	CAS_R1_Term	111

Primer sequences

Primer Name	Primer Sequence (5' > 3')	
CAS_R1_Term	TCGTGGTATCGTTATGCGCC	
Capzb_47322_F	GACTCGAACAGAAAGGCATCC	
Capzb_47322_R	GATCCAGGCTGTCCATAGGTG	
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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Relevant publications

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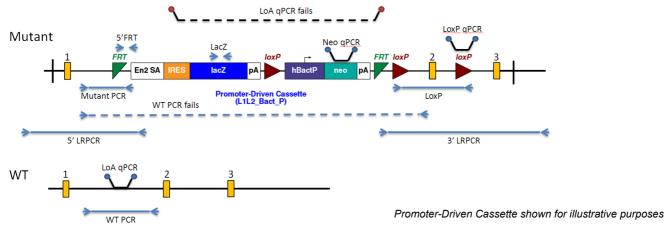
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Southern Blot	na	TV Backbone Assay	pass	5' LR-PCR	na
Loss of WT Allele (LOA) qPCR	na	Homozygous Loss of WT Allele (LOA) SR-PCR	na	Neo Count (qPCR)	pass
LacZ SR-PCR	pass	5' Cassette Integrity	pass	Neo SR-PCR	na
Mutant Specific SR-PCR	pass	LoxP Confirmation	pass	3' LR-PCR	na
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