

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

Gene: Mfrp

Colony prefix: MDCC

ESC clone ID: EPD0555\_1\_E10

WT PCR

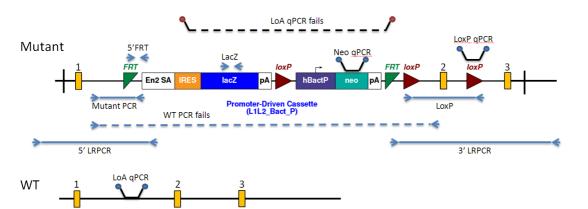
Allele: Mfrp<sup>tm2a(KOMP)Wtsi</sup>

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/81518 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					

This technical data sheet and information ("Datasheet") is supplied by Genome Research Limited ("GRL").



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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/martsearch/ikmc\_project/81518

# 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	Mfrp_F2	Mfrp_R2	238
Standard PCR	Mutant	Mfrp_F2	CAS_R1_Term	136
Standard PCR	Cassette	LacZ_2_small_F	LacZ_2_small_R	108

#### **Primer sequences**

Primer Name	Primer Sequence (5' > 3')	
CAS_R1_Term	TCGTGGTATCGTTATGCGCC	
LacZ_2_small_F	ATCACGACGCGCTGTATC	
LacZ_2_small_R	ACATCGGGCAAATAATATCG	
Mfrp_F2	TACAGGCCCTCTCCTCAGGT	
Mfrp_R2	TGTAAGAAAGCCAGGGAGGA	

#### **Reaction setup**

Reagent	μί
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	-
6	72°C	5 min
7	12°C	forever

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#### **Relevant publications**

Ryder, E., Doe, B., Gleeson, D., Houghton, R., Dalvi, P., Grau, E., Ramirez-Solis, R. (2013). Rapid conversion of EUCOMM/KOMP-CSD alleles in mouse embryos using a cell-permeable Cre recombinase. Transgenic research. 23(1), 177–185.

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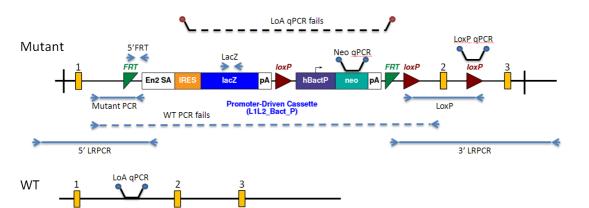
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