

**Gene:** Rreb1

**Colony prefix:** MHFU

**ESC clone ID:** EPD0486\_4\_C04

**Allele:** *Rreb1<sup>tm1a(EUCOMM)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/69596](http://www.mousephenotype.org/martsearch_ikmc_project/martsearch/ikmc_project/69596)  
Details on how to determine the floxed exon can be found at <http://www.i-dcc.org/kb/entry/21/>

**Mouse QC information**



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

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## Southern blot confirmation:

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## 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](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	Rreb1_81122_F	Rreb1_81122_R	352
Standard PCR	Mutant	Rreb1_81122_F	CAS_R1_Term	192
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
Rreb1_81122_F	AAGCCCAAAGTCCTCGTGTC
Rreb1_81122_R	GCAGTCCCTCCTCCAGTTTG

## Reaction setup

Reagent	µl
DNA (~50-100 ng)	1
10x Buffer	2
MgCl <sub>2</sub> (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
ddH <sub>2</sub> O	15.2
<b>Total</b>	<b>20</b>

## 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, Tfr; 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	TGGGCACAACAGACAATCGGCT G

Reactions are performed in a 10µl volume using an Applied Biosystems 7900HT Fast Real-Time PCR System or Applied Biosystems Viiia7 with DNA prepared using the Sample-to-SNP™ kit (Applied Biosystems) from mouse ear biopsies. GTXpress™ buffer is also used (Applied Biosystems).

Reagent	µl
2x GTXpress™ buffer	5
20x target assay	0.5
ddH2O	3
Tfr endogenous 20x assay	0.5
DNA	1

### Amplification conditions

Step	Conditions	Time
1	95°C	20 sec
2	95°C	10 sec
3	60°C	30 sec
4	Go to '2' + 34 cycles	-

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## Relevant publications

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Bradley, A., Anastassiadis, K., Ayadi, A., Battey, J.F., Bell, C., Birling, M.-C., Bottomley, J., Brown, S.D., Bürger, A., Bult, C.J., et al. (2012). The mammalian gene function resource: the international knockout mouse consortium. *Mamm Genome* 23, 580–586.

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Pettitt, S.J., Liang, Q., Rairdan, X.Y., Moran, J.L., Prosser, H.M., Beier, D.R., Lloyd, K.C., Bradley, A., and Skarnes, W.C. (2009). Agouti C57BL/6N embryonic stem cells for mouse genetic resources. *Nat Methods* 6, 493–495.

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**Colony prefix:** MHFU

**ESC clone ID:** EPD0486\_4\_C04

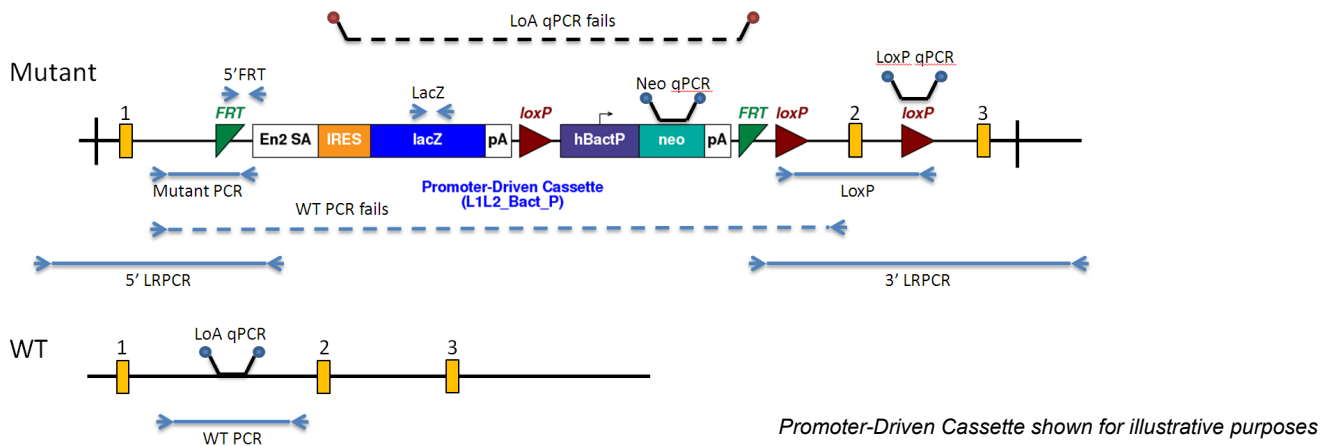
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Details on how to determine the floxed exon can be found at <http://www.i-dcc.org/kb/entry/21/>

**Mouse QC information**



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

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IKMC allele types:

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MGP mouse quality control tests :

<http://www.i-dcc.org/kb/25/>

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Standard PCR	Mutant	Rreb1_81122_F	CAS_R1_Term	192
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
Rreb1_81122_F	AAGCCCAAAGTCCTCGTGTC
Rreb1_81122_R	GCAGTCCCTCCTCCAGTTTG

## Reaction setup

Reagent	µl
DNA (~50-100 ng)	1
10x Buffer	2
MgCl <sub>2</sub> (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
ddH <sub>2</sub> O	15.2
<b>Total</b>	<b>20</b>

## Amplification conditions

Step	Conditions	Time
1	94°C	5 min
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Reactions are performed in a 10µl volume using an Applied Biosystems 7900HT Fast Real-Time PCR System or Applied Biosystems Vii7 with DNA prepared using the Sample-to-SNP™ kit (Applied Biosystems) from mouse ear biopsies. GTXpress™ buffer is also used (Applied Biosystems).

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Tfr endogenous 20x assay	0.5
DNA	1

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