

**Gene:** Rapgef1

**Colony prefix:** MDCW

**ESC clone ID:** EPD0079\_5\_G07

**Allele:** Rapgef1<sup>tm1a(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/25008](http://www.mousephenotype.org/martsearch_ikmc_project/martsearch/ikmc_project/25008)  
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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## 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 | Cassette | LacZ_2_small_F  | LacZ_2_small_R  | 108                     |
| Standard PCR | Mutant   | Rapgef1_43806_F | CAS_R1_Term     | 139                     |
| Standard PCR | Wildtype | Rapgef1_43806_F | Rapgef1_43806_R | 355                     |

## Primer sequences

| Primer Name     | Primer Sequence (5' > 3') |
|-----------------|---------------------------|
| CAS_R1_Term     | TCGTGGTATCGTTATGCGCC      |
| LacZ_2_small_F  | ATCACGACGCGCTGTATC        |
| LacZ_2_small_R  | ACATCGGGCAAATAATATCG      |
| Rapgef1_43806_F | GCCTGGGCACTTCCTGATAC      |
| Rapgef1_43806_R | CACTGGACAGGCTGCGATAC      |

## 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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## Relevant publications

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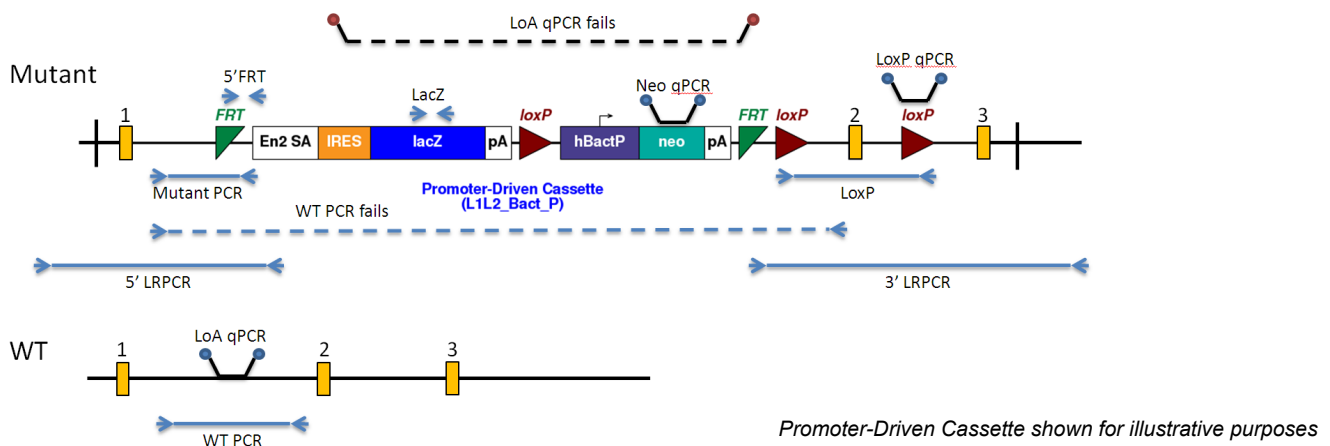
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