

**Gene:** Maneal

**Colony prefix:** MBDB

**ESC clone ID:** EPD0026\_3\_E12

**Allele:** Maneal<sup>Itm1a(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/38315](http://www.mousephenotype.org/martsearch_ikmc_project/martsearch/ikmc_project/38315)  
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>        | fail |
| <b>Loss of WT Allele (LOA) qPCR</b> | pass | <b>Homozygous Loss of WT Allele (LOA) SR-PCR</b> | pass | <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:

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/38315](http://www.mousephenotype.org/martsearch_ikmc_project/martsearch/ikmc_project/38315)

## 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 | Wildtype | Maneal_36741_F | Maneal_36741_R | 161                     |
| Standard PCR | Mutant   | Maneal_36741_F | CAS_R1_Term    | 127                     |

## Primer sequences

| Primer Name    | Primer Sequence (5' > 3') |
|----------------|---------------------------|
| CAS_R1_Term    | TCGTGGTATCGTTATGCGCC      |
| LacZ_2_small_F | ATCACGACGCGCTGTATC        |
| LacZ_2_small_R | ACATCGGGCAAATAATATCG      |
| Maneal_36741_F | GGGTGAGACAGGATTCCCAG      |
| Maneal_36741_R | GCCTGGACAGAATAGCCCAC      |

## 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<br>G |

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).

| 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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### 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 Maneal 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, Tfr; Applied Biosystems part #4458366). Reference DNA controls of known genotypes should also be included to facilitate correct analysis.

#### Primers for LoA qPCR assay

| Primer type | Assay Name | Forward Primer Seq.     | Reverse Primer Seq.              | Probe Primer Seq.      |
|-------------|------------|-------------------------|----------------------------------|------------------------|
| LoA         | MANEAL_WT  | TGGACAGAATAGCCCACATCCTA | ATGAGCAAACGCATAAAACAATA<br>AGGAG | ATGGGACATATACAAAGATAAT |

#### 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

- 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.
- Ryder, E., Gleeson, D., Sethi, D., Vyas, S., Miklejewska, E., Dalvi, P., Habib, B., Cook, R., Hardy, M., Jhaveri, K., et al. (2013). Molecular Characterization of Mutant Mouse Strains Generated from the EUCOMM/KOMP-CSD ES Cell Resource. *Mamm Genome*, 24, 286–294.
- White, J.K., Gerdin, A.-K., Karp, N.A., Ryder, E., Buljan, M., Bussell, J.N., Salisbury, J., Clare, S., Ingham, N.J., Podrini, C., et al. (2013). Genome-wide Generation and Systematic Phenotyping of Knockout Mice Reveals New Roles for Many Genes. *Cell* 154, 452–464.
- Ryder, E., Wong, K., Gleeson, D., Keane, T.M., Sethi, D., Vyas, S., Wardle-Jones, H., Bussell, J.N., Houghton, R., Salisbury, J., et al. (2013). Genomic analysis of a novel spontaneous albino C57BL/6N mouse strain. *Genesis* 51, 523–528.
- 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.
- Birling, M.-C., Dierich, A., Jacquot, S., Héroult, Y., and Pavlovic, G. (2011). Highly-efficient, fluorescent, locus directed Cre and flopo deleter mice on a pure C57BL/6N genetic background. *Genesis*.
- Skarnes, W.C., Rosen, B., West, A.P., Koutsourakis, M., Bushell, W., Iyer, V., Mujica, A.O., Thomas, M., Harrow, J., Cox, T., et al. (2011). A conditional knockout resource for the genome-wide study of mouse gene function. *Nature* 474, 337–342.
- 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.
- Liang, Q., Conte, N., Skarnes, W.C., and Bradley, A. (2008). Extensive genomic copy number variation in embryonic stem cells. *Proc Natl Acad Sci U S A* 105, 17453–17456.
- Farley, F.W., Soriano, P., Steffen, L.S., and Dymecki, S.M. (2000). Widespread recombinase expression using FLPeR (flipper) mice. *Genesis* 28, 106–110.

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