

Gene: Mill2

Colony prefix: DAQT

Allele: *Mill2^{em1 (IMPC)Wtsi}*

Allele type: Crispr/Cas9 mediated deletion

Anticipated mutation: More than 50% CDS deleted

Allele information:

Further information about the allele can be found on the 'International Mouse Phenotyping Consortium' (IMPC) web site at <http://www.mousephenotype.org>



Mouse QC information

| | | | |
|-------------------------------------|------|-------------------------------------|------|
| Loss of WT Allele (LOA) qPCR | Pass | Mutation Sequence confirmed | Pass |
| Mutant Specific SR-PCR | Pass | Off-target analysis complete | na |

Mutant Allele sequence:

```
ATGTTCTTTGCCTAGGAAGAAAGTTGCAGAGCTCTTGAGTATTTTGCTTCTTTGGATGTTAACAAACATCTCAAT
GATTGTATGAAATGCAATTGTCTCAAAAAGACTGAGTAGAACCCATGTTTATTTCCATTTTCTCAGGGTCTCCAGCCG
GACTACAGATTCAAACGCAAGCTACCCAACCCATGTTCCATATCCAAGGTCCTTCTCCTGAAGCTTAGCTCTTTGT
TTTTGTTTTTAAAGATTTATTTATTTAATGTATATGAGTACTCTGTCTTCATGCACACCAGAAGAAAGCA
```

Deletion size (bp): 302

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Guide RNAs used in initial experiment

| Sequence | Chr | Chr Start | Chr End |
|-------------------------|-----|-----------|----------|
| CCAGCCCTTGGGTGAAGGTTATT | 7 | 18855744 | 18855766 |
| CCCACTCTGCGCTATAATGTCA | 7 | 18855944 | 18855966 |
| AGAAACGGTCTGCAGGATACAGG | 7 | 18856660 | 18856682 |
| CCATGGAGCCGGACTACAGATTC | 7 | 18856824 | 18856846 |

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Genotyping by end-point PCR

These mice may be genotyped through a combination of separate PCR reactions that detect 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. In addition to the expected product, the mutant assay may also amplify the endogenous wild type sequence which will appear as a larger band on an agarose gel. The presence of this extra band will depend on the size of the original deletion.

PCRs primer pairs and expected size bands

| Assay Type | Assay | Forward Primer | Reverse Primer | Expected Size Band (bp) |
|--------------|-----------|----------------|----------------|-------------------------|
| Standard PCR | Wild type | Mill2_DF1 | Mill2_ER1 | 386 |
| Standard PCR | Wild type | Mill2_EF1 | Mill2_DR1 | 446 |
| Standard PCR | Mutant | Mill2_DF1 | Mill2_DR1 | 214 |

Primer sequences

| Primer Name | Primer Sequence (5' > 3') |
|-------------|---------------------------|
| Mill2_DF1 | TGCCTAGGAAGAAAGTTGCAG |
| Mill2_ER1 | TCTCAGACCCCTCCAGAGAA |
| Mill2_EF1 | CTAGAGGCTTCTGGCACCTG |
| Mill2_DR1 | GAGCTAAGCTTCAGGAGAAGGA |

Reaction setup

| Reagent | µl |
|---------------------------|-------|
| DNA (~50-100 ng) | 1 |
| 10x Buffer | 1.5 |
| MgCl ₂ (50 mM) | 0.45 |
| Platinum Taq (Invitrogen) | 0.15 |
| dNTPs (100 mM) | 0.15 |
| Primer 1 (10 µM) | 0.3 |
| Primer 2 (10 µM) | 0.3 |
| ddH ₂ O | 11.15 |
| Total | 15 |

Amplification conditions

| Step | Conditions | Time |
|------|-----------------------|----------|
| 1 | 94°C | 5 min |
| 2 | 94°C | 30 sec |
| 3 | 58°C | 30 sec |
| 4 | 72°C | 1:30 sec |
| 5 | Go to '2' + 34 cycles | - |
| 6 | 72°C | 5 min |
| 7 | 12°C | forever |

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

Primers for LoA qPCR assay

| Gene | Source | Forward Primer Seq. | Reverse Primer Seq. | Probe Primer Seq. |
|-------|-------------------|--------------------------------|---------------------|--------------------|
| Mill2 | Life Technologies | GAGACCTGTGCAAGAGAGAC TAACAA | GCCATCATCCCCCTGAGTT | CTGTTGAAGGTGGAAGAG |

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-SNPTM 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 |
| Tfrc 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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Links to information and frequently asked questions

MGP mouse phenotype data:

<http://www.mousephenotype.org>

Useful publications

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.

Mali P, Yang L, Esvelt KM, et al (2013) RNA-guided human genome engineering via Cas9. *Science* 339:823–6. doi: 10.1126/science.1232033

Jinek M, Chylinski K, Fonfara I, et al (2012) A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science* 337:816–21. doi: 10.1126/science.1225829

Cong L, Ran FA, Cox D, et al (2013) Multiplex genome engineering using CRISPR/Cas systems. *Science* 339:819–23. doi: 10.1126/science.1231143

Singh P, Schimenti JC, Bolcun-Filas E (2014) A Mouse Geneticist's Practical Guide to CRISPR Applications. *Genetics* 194:169771–. doi: 10.1534/genetics.114.169771

Brandl C, Ortiz O, Röttig B, et al (2015) Creation of targeted genomic deletions using TALEN or CRISPR/Cas nuclease pairs in one-cell mouse embryos. *FEBS Open Bio* 5:26–35. doi: 10.1016/j.fob.2014.11.009

Zhou J, Wang J, Shen B, et al (2014) Dual sgRNAs facilitate CRISPR/Cas9 mediated mouse genome targeting. *FEBS J*. doi: 10.1111/febs.12735

Kraft K, Geuer S, Will AJ, et al (2015) Deletions, Inversions, Duplications: Engineering of Structural Variants using CRISPR/Cas in Mice. *Cell Rep*. doi: 10.1016/j.celrep.2015.01.016

Shen B, Zhang J, Wu H, et al (2013) Generation of gene-modified mice via Cas9/RNA-mediated gene targeting. *Cell Res* 23:720–3. doi: 10.1038/cr.2013.46

Wang H, Yang H, Shivalila CS, et al (2013) One-step generation of mice carrying mutations in multiple genes by CRISPR/Cas-mediated genome engineering. *Cell* 153:910–8. doi: 10.1016/j.cell.2013.04.025

Yang H, Wang H, Shivalila CS, et al (2013) One-Step Generation of Mice Carrying Reporter and Conditional Alleles by CRISPR/Cas-Mediated Genome Engineering. *Cell* 154:1370–1379. doi: 10.1016/j.cell.2013.08.022

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