Wellcome Sanger Institute
Wellcome Genome Campus
Hinxton Cambridge CB10 1SA, U.K.

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

**Gene:** Dnase1l2

**Colony prefix:** *MAYG*

**ESC clone ID:** *EPD0296_2_A10*

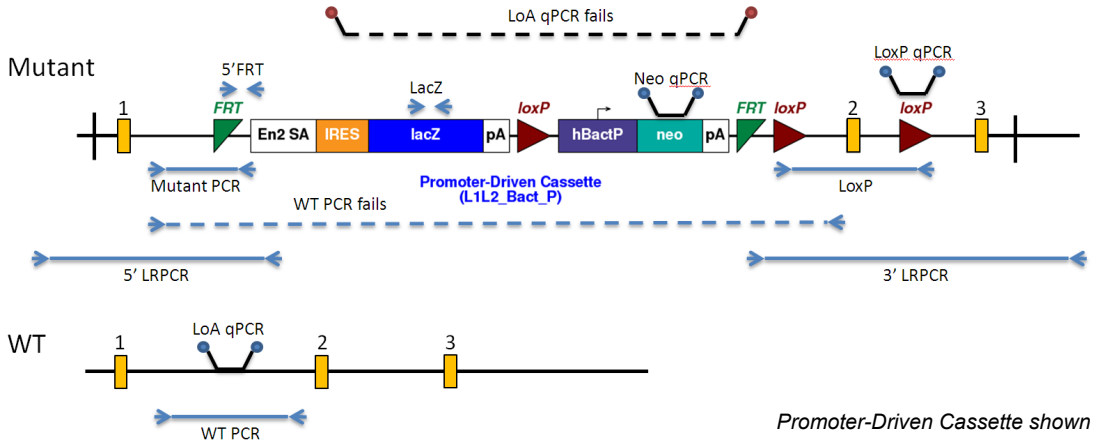
**Allele:** *Dnase1l2*$^{tm1(KOMP)Wtsi}$

**Allele type:** *Deletion*

## 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/33505
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** | na | **Neo SR-PCR** | na |
| **Mutant Specific SR-PCR** | pass | **LoxP Confirmation** | na | **3' LR-PCR** | na |
| **Genotyping Comment** | | | | | |


Report Generated on: 02-JUL-2020 15:34:24

Wellcome Sanger Institute
Wellcome Genome Campus
Hinxton Cambridge CB10 1SA, U.K.

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

## 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/33505

## 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.


Report Generated on: 02-JUL-2020 15:34:24

Wellcome Sanger Institute
Wellcome Genome Campus
Hinxton Cambridge CB10 1SA, U.K.

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

## PCRs primer pairs and expected size bands

| Assay Type | Assay | Forward Primer | Reverse Primer | Expected Size Band (bp) |
|---|---|---|---|---|
| Standard PCR | Wildtype | Dnase1l2_F | Dnase1l2_R | 399 |
| Standard PCR | Mutant | Dnase1l2_F | CAS_R1_Term | 309 |
| Standard PCR | Cassette | LacZ_2_small_F | LacZ_2_small_R | 108 |

## Primer sequences

| Primer Name | Primer Sequence (5' > 3') |
|---|---|
| CAS_R1_Term | TCGTGGTATCGTTATGCGCC |
| Dnase1l2_F | TGAGTGCTTTAAAATCAGAGC |
| Dnase1l2_R | GTGGCTCCCATGACCCCCAGT |
| LacZ_2_small_F | ATCACGACGCGCTGTATC |
| LacZ_2_small_R | ACATCGGGCAAATAATATCG |

## Reaction setup

| Reagent | µl |
|---|---|
| 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 µM) | 0.4 |
| Primer 2 (10 µM) | 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 |


Report Generated on: 02-JUL-2020 15:34:24

Wellcome Sanger Institute
Wellcome Genome Campus
Hinxton Cambridge CB10 1SA, U.K.

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

## 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 Dnase1l2 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***

| Primer type | Assay Name | Forward Primer Seq. | Reverse Primer Seq. | Probe Primer Seq. |
|---|---|---|---|---|
| LoA | LacZ | GGAGTGCGATCTTCCTGAGG | CGCATCGTAACCGTGCATC | CGATACTGTCGTCGTCCCCTCAAACTG |

***Reaction setup***

Reaction setup and amplification conditions are the same as those used for the neo cassette qPCR assay.


Report Generated on: 02-JUL-2020 15:34:24

Wellcome Sanger Institute
Wellcome Genome Campus
Hinxton Cambridge CB10 1SA, U.K.

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

## 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., Hérault, Y., and Pavlovic, G. (2011). Highly-efficient, fluorescent, locus directed Cre and flpo 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.


Report Generated on: 02-JUL-2020 15:34:24