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

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

Gene: Mroh7

Colony prefix: *MDLM*

ESC clone ID: *EPD0156_6_F07*

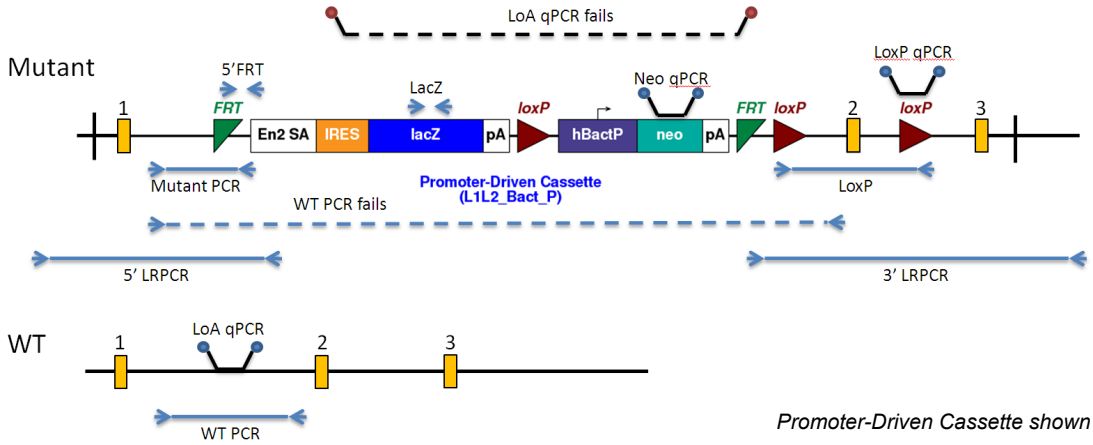
Allele: *Mroh7$^{tm1a(EUCOMM)Wtsi}$*

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/34301
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**	na	**5' LR-PCR**	na
Loss of WT Allele (LOA) qPCR	na	**Homozygous Loss of WT Allele (LOA) SR-PCR**	na	**Neo Count (qPCR)**	na
LacZ SR-PCR	na	**5' Cassette Integrity**	na	**Neo SR-PCR**	na
Mutant Specific SR-PCR	na	**LoxP Confirmation**	na	**3' LR-PCR**	na
Genotyping Comment					

Report Generated on: 19-MAY-2020 21:36:50

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/34301

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: 19-MAY-2020 21:36:50

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	Gm1027_47191_F	Gm1027_47191_R	209
Standard PCR	Mutant	Gm1027_47191_F	CAS_R1_Term	151
Standard PCR	Cassette	LacZ_2_small_F	LacZ_2_small_R	108

Primer sequences

Primer Name	Primer Sequence (5' > 3')
CAS_R1_Term	TCGTGGTATCGTTATGCGCC
Gm1027_47191_F	CAGGGAAAGGGAGAAGGAAG
Gm1027_47191_R	GAGGGAACAGGACTCTGCTG
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: 19-MAY-2020 21:36:50

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: 19-MAY-2020 21:36:50

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

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

Gene: Mroh7

Colony prefix: *MDLM*

ESC clone ID: *EPD0156_6_F07*

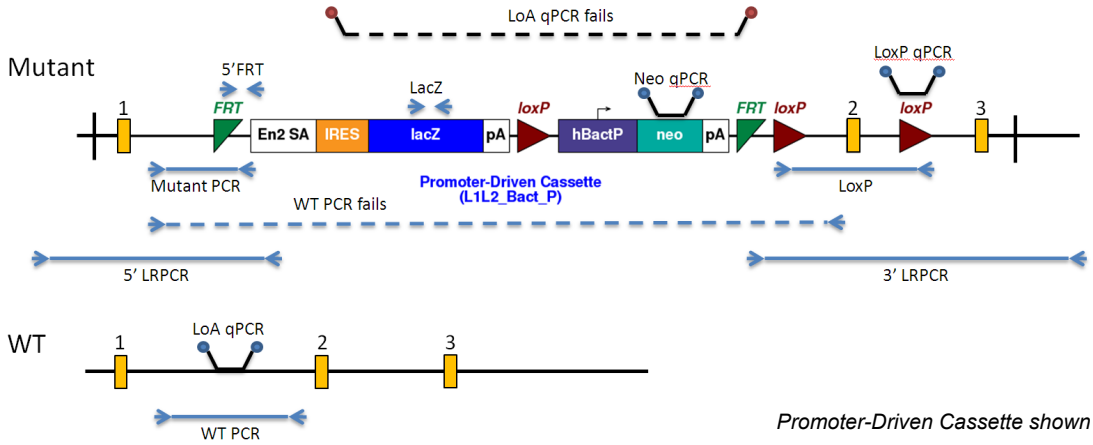
Allele: *Mroh7$^{tm1a(EUCOMM)Wtsi}$*

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/34301
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**	pass	**Neo SR-PCR**	na
Mutant Specific SR-PCR	pass	**LoxP Confirmation**	pass	**3' LR-PCR**	na
Genotyping Comment					

Report Generated on: 19-MAY-2020 21:36:50

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/34301

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: 19-MAY-2020 21:36:50

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	Gm1027_47191_F	Gm1027_47191_R	209
Standard PCR	Mutant	Gm1027_47191_F	CAS_R1_Term	151
Standard PCR	Cassette	LacZ_2_small_F	LacZ_2_small_R	108

Primer sequences

Primer Name	Primer Sequence (5' > 3')
CAS_R1_Term	TCGTGGTATCGTTATGCGCC
Gm1027_47191_F	CAGGGAAAGGGAGAAGGAAG
Gm1027_47191_R	GAGGGAACAGGACTCTGCTG
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: 19-MAY-2020 21:36:50

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: 19-MAY-2020 21:36:50