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

MGPgenotyping@sanger.ac.uk
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Gene: Mir96

Colony prefix: TCAA

ESC clone ID: BEPD0019_D04

Allele: *Mir96*$^{Tm2.1WTSI}$

Allele type: *SNP (post Flp, with no selection cassette)*

Mutation Sequence:

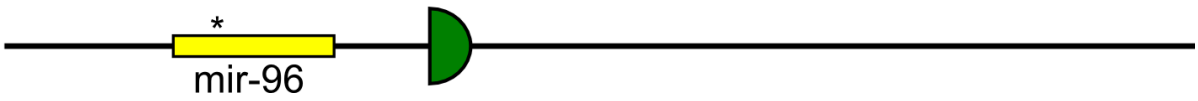
CCAGTACCATCTGCTTGGCCGATTTTG[G/A]CACTAGCACATTTTTGCTTGTGTCTCTCCGCTGTGAGCAATCATG
TGTAGTGCCAATATGGGAAAAGCGGGCTGCTGC

Allele information:

Further information about the allele can be found on the 'International Mouse Phenotyping Consortium' (IMPC) web site at https://beta.mousephenotype.org/data/alleles/MGI:3619440/tm2.1%28MGP%29Wtsi/

Mouse QC information

Post flp-excision allele



Southern Blot	na	**TV Backbone Assay**	na	**5' LR-PCR**	na
Loss of WT Allele (LOA) qPCR	Yes	**Neo Count (qPCR)**	na	**Mutation sequence confirmed**	Inferred from parent colony
Mutant Specific SR-PCR	Inferred from parent colony	**3' LR-PCR**	na		
Genotyping Comment					

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Southern blot confirmation:

Southern blots are not routinely performed at the Sanger Institute due to throughput constraints.

Links to information and frequently asked questions about the EUCOMM/KOMP alleles and MGP projects

MGP mouse phenotype data:
http://www.mousephenotype.org

MGP mouse quality control tests :
http://www.i-dcc.org/kb/25/

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Genotyping Information

PCRs primer pairs and expected size bands

Assay Type	Assay	Forward Primer	Reverse Primer	Expected Size Band (bp)
Standard PCR	Mutant	Mir96_F2	CAS_R1_Term	587

Primer sequences

Primer Name	Primer Sequence (5' > 3')
CAS_R1_Term	TCGTGGTATCGTTATGCGCC
Mir96_F2	CTCACCCCTTTCTGCCTAGA

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

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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 WT Mir96 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	Mir96_WT	CACTAGTGGGTGGATGGAAAGG	TGGAGGGCTGGGAATGTTC	CACAACTGAGCCTTC

Reactions are performed in a 10µl volume using an Applied Biosystems 7900HT Fast Real-Time PCR System or Applied Biosystems Viia7 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
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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Genotyping by SNP qPCR Assay (gene-specific assay)

SNP assays consist of unlabeled forward and reverse primers, and a mix of two hydrolysis probes designed to either the wild type or mutant allele sequences. The probes are labelled with different fluorescent dyes which allow the calling of genotypes as cluster plots based on the levels of fluorescence after an end-point qPCR reaction.

Primers for SNP qPCR assay

Assay	Assay Name	Forward Primer Seq.	Reverse Primer Seq.	Probe Primer Seq.
SNP	Mir96_allele-34	GGGCCTGTTCCAGTACCATCT	GCGGAGAGACACAAGCAAAAA	[VIC]ATTTTGACACTAGCAC, [FAM]TTGGCCGATTTTGGCA

Reactions are performed in a 10µl volume using an Applied Biosystems 7900HT Fast Real-Time PCR System or Applied Biosystems Viia7 with DNA prepared using the Sample-to-SNP*TM* kit (Applied Biosystems) from mouse ear biopsies. GTXpress*TM* buffer is also used (Applied Biosystems).

Reagent	µl
2x GTXpress*TM* buffer	5
40x target assay	0.25
ddH2O	3.75
DNA	1

Amplification conditions

Step	Conditions	Time
1 (pre-read)	60°C	30 sec
2	95°C	10 min
3	95°C	15 sec
4	60°C	1 min
5	Go to '3' + 34 cycles	
6 (post-read)	60°C	30 sec

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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.

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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.

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