Wellcome Trust Sanger Institute
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MGPgenotyping@sanger.ac.uk
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Gene: Trappc9

Colony prefix: *MMAQ*

ESC clone ID: EPD0596_4_E10

Allele: *Trappc9$^{tm1c(EUCOMM)wtsi}$*

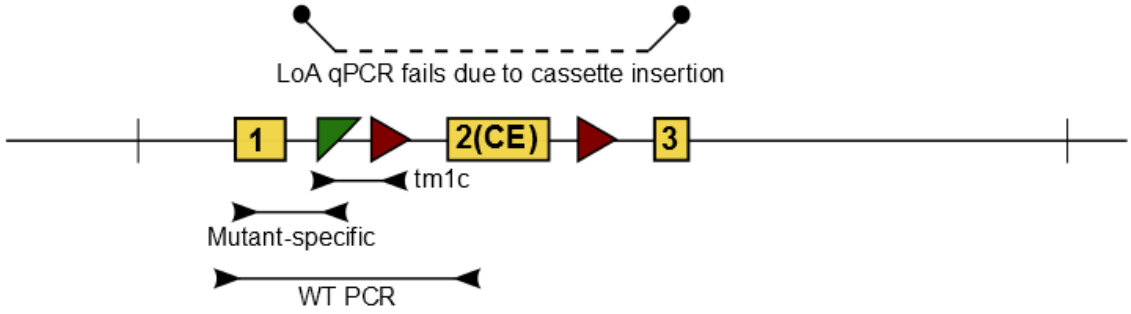
Allele type: *Conditional allele (post-Flp)*

Allele information:

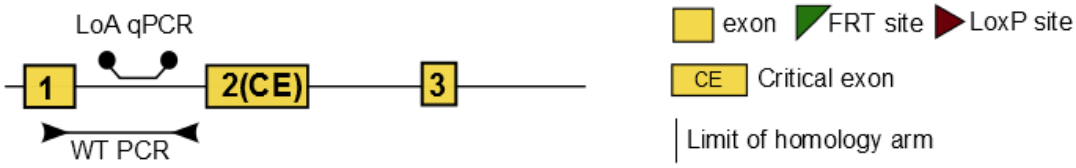
Further information about the allele can be found on the IKMC web site at http://www.mousephenotype.org/martsearch_ikmc_project/martsearch/ikmc_project/46714. Details on how to determine the floxed exon can be found at http://www.knockoutmouse.org/kb/entry/21/

Mouse QC information

Mutant allele



WT allele



Southern Blot	na	**TV Backbone Assay**	N/A	**5' LR-PCR**	N/A
Loss of WT Allele (LOA) qPCR	pass	**Homozygous Loss of WT Allele (LOA) SR-**	pass	**Neo Count (qPCR)**	N/A
LacZ SR-PCR	N/A	**5' Cassette Integrity**	N/A	**Neo SR-PCR**	N/A
Mutant Specific SR-PCR	pass	**LoxP Confirmation**	N/A	**3' LR-PCR**	N/A
Genotyping Comment					

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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 IKMC web site at
http://www.knockoutmouse.org/martsearch/project/69506

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

General targeting strategies:
http://www.knockoutmouse.org/about/targeting-strategies

MGP mouse phenotype data:
http://www.sanger.ac.uk/mouseportal/

IKMC allele types:
http://www.knockoutmouse.org/kb/entry/89/

MGP mouse quality control tests :
http://www.knockoutmouse.org/kb/25/

Allele conversion guide - genotyping tm1b, tm1c and tm1d mice:
http://www.knockoutmouse.org/kb/entry/105/

How the "critical" exon is decided:
http://www.knockoutmouse.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.

Please note that for tm1c mice the mutant allele will still amplify a band in the WT assay, but the size will be different to the native WT product (exact size difference is allele-specific).

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PCRs primer pairs and expected size bands

Assay Type	Assay	Forward Primer	Reverse Primer	Expected Size Band (bp)
Standard PCR	Wildtype	Trappc9_176362_F	Trappc9_176362_R	534
Standard PCR	Mutant	Trappc9_176362_F	CAS_R1_Term	397
Standard PCR	Cassette	Tm1c_F	Tm1c_R	218

Primer sequences

Primer Name	Primer Sequence (5' > 3')
CAS_R1_Term	TCGTGGTATCGTTATGCGCC
Tm1c_F	AAGGCGCATAACGATACCAC
Tm1c_R	CCGCCTACTGCGACTATAGAGA
Trappc9_176362_F	CTGGGCGGTCTCTTAGCTTC
Trappc9_176362_R	TTGAAAGTGCTGGTTGCCTC

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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Relevant publications

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. Mammalian Genome. Doi: 10.1007/s00335-013-9467-x

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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.

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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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