

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

Gene: Nfya

Colony prefix: MDFA

ESC clone ID:

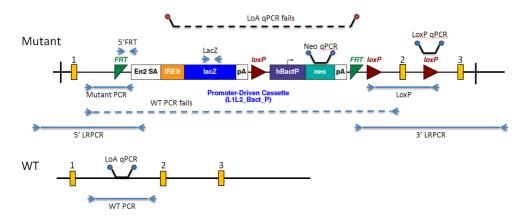
Allele: NfyaGt(EUCJ0004f10)Hmgu

Allele type: Knockout First, Reporter-tagged insertion with conditional potential

Allele information:

Details on how to determine the floxed exon can be found at http://www.knockoutmouse.org/kb/entry/21/

Mouse QC information



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

This technical data sheet and information ("Datasheet") is supplied by Genome Research Limited ("GRL").



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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

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.

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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	Nfya_GT_F	Nfya_GT_R	632
Standard PCR	Mutant	Nfya_GT_F	Nfya_GT_Mut_R	

Primer sequences

Primer Name	Primer Sequence (5' > 3')	
Nfya_GT_F	GAGCATTTCTGGGCATTTCAGCTT	
Nfya_GT_Mut_R	GTCCTCCGATTGACTGAGTCGC	
Nfya_GT_R	CTGTATTTTACCGCTTCCACTAGC	

Reaction setup

Reagent	μΙ
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

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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