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

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

Gene: Nek3

Colony prefix: PMBT

ESC clone ID: EPD0430_2_G09

Allele: Nek3$^{tm1b(EUCOMM)Wtsi}$

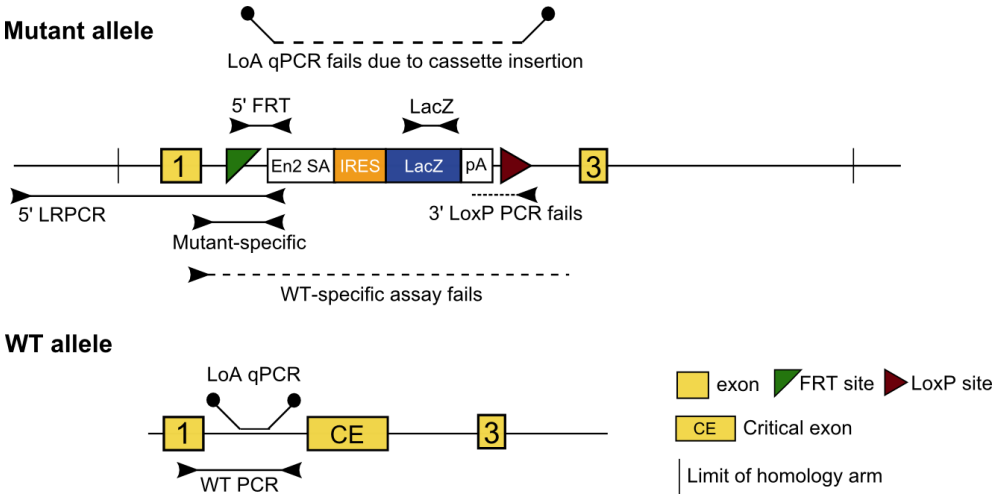
Allele type: *Reporter-tagged deletion allele (post-cre)*

Allele information:

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

Mouse QC information

Promoter Driven:



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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/78751

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	Wild type	Nek3_49254_F	Nek3_49254_R	566
Standard PCR	Mutant	Nek3_49254_F	CAS_R1_Term	331
Standard PCR	Cassette	LacZ_2_small_F	LacZ_2_small_R	108

Primer sequences

Primer Name	Primer Sequence (5' > 3')
CAS_R1_Term	TCGTGGTATCGTTATGCGCC
LacZ_2_small_F	ATCACGACGCGCTGTATC
LacZ_2_small_R	ACATCGGGCAAATAATATCG
Nek3_49254_F	TGGTCTTAGCCATTGGGAGG
Nek3_49254_R	CGAACATGCAGAAGTCAGGTG

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 using universal copy number qPCR assays designed to the selection cassette

The cassette qPCR assays use a hydrolysis probe assay (eg Applied Biosystems TaqMan technology) to determine genotype via the copy number of the selection cassette in a sample. Homozygotes will possess two copies, heterozygotes one copy and wild type mice will show no amplification when compared to known homozygote controls.

These FAM®-labeled assays are multiplexed with a VIC® labeled endogenous control assay (for example TaqMan® Copy Number Reference Assay, Mouse, Tfrc; Applied Biosystems part #4458366).

Please note that these assays are not gene-specific – other information should be used in conjunction with the universal cassette assays (for example the mutant-specific srPCR) when confirming the gene identity.

Primer type	Assay Name	Forward Primer Seq.	Reverse Primer Seq.	Probe Primer Seq.
Cassette	LacZ_reg	GGAGTGCGATCTTCCTGAGG	CGCATCGTAACCGTGCATC	CGATACTGTCGTCGTCCCCTCAAACTG

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
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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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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