Gene: Adnp

Colony prefix: TAGX

ESC clone ID: BEPD0199_C02
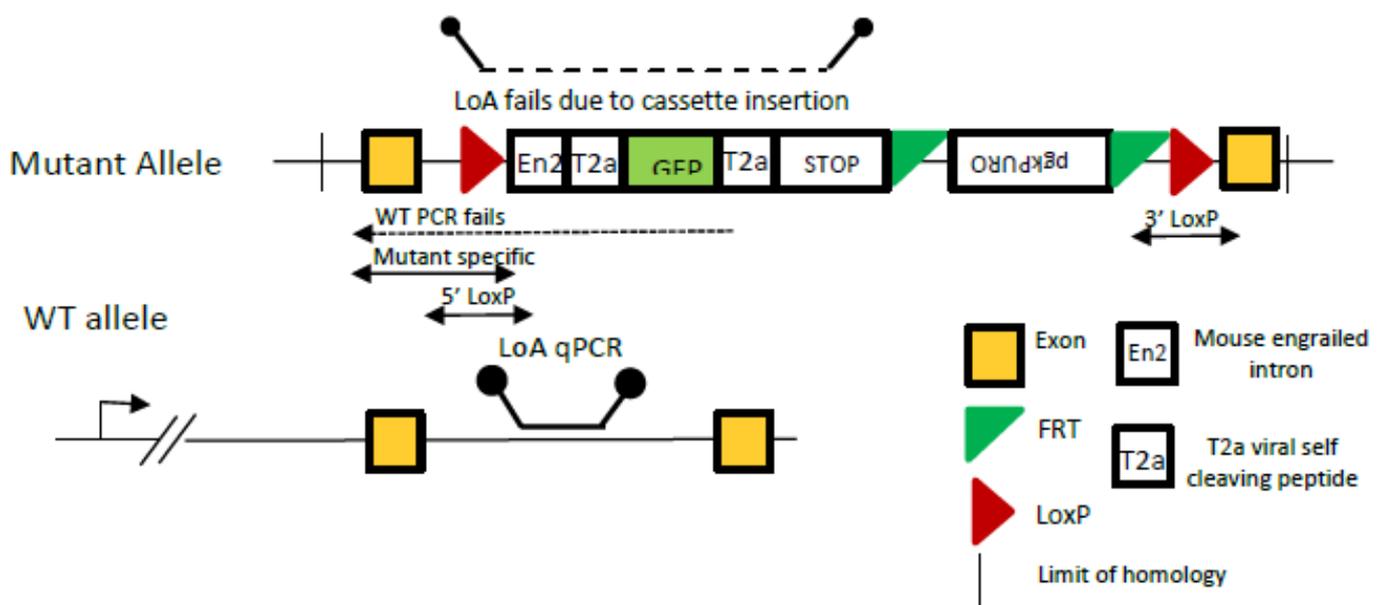
Allele: $Adnp^{2a\_GFP\_H2b}$

Allele type: *Artificial Intron KI GFP STOP reporter, KO first, Cre induction to recover WT activity.*

Allele information:

Further information about the allele can be found on the 'International Mouse Phenotyping Consortium' (IMPC) web site at http://www.mousephenotype.org

Details on how to determine the deleted exon can be found at http://www.i-dcc.org/kb/entry/21/

Report Generated on: 14th July 2021

Genome Research Limited
Registered Office 215 Euston Road London NW1 2BE. A company registered in England No. 2742969 and a charity No. 1021457

Page 1 of 6

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.mousephenotype.org/about-ikmc/targeting-strategies

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.infrafrontier.eu/sites/infrafrontier.eu/files/upload/public/pdf/Resources%20and%20Services/eucomm_komp-csd_allele_conversion_guide_v3a_2016.pdf

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.

Genome Research Limited
Registered Office 215 Euston Road London NW1 2BE. A company registered in England No. 2742969 and a charity No. 1021457

Page 2 of 6

PCRs primer pairs and expected size bands

Assay Type	Assay	Forward Primer	Reverse Primer	Expected Size Band (bp)
Standard PCR	Wildtype	Adnp_seq_F	Adnp_seq_R	286
Standard PCR	Mutant	Adnp_seq_R	SG_Mut_R2	134
Standard PCR	5'LoxP	SG_5LOXP_F2	SG_5LOXP_R	200
Standard PCR	3'LoxP	SG_3LOXP_F	SG_3LOXP_R2	239

Primer sequences

Primer	Primer Sequence (5' > 3')
Adnp_seq_F	TTGGGAACAAGGAGATCTGG
Adnp_seq_R	AGGCTCTCAGCTAGGGAAGG
SG_Mut_R2	AATGTGTTGCAACGAACAGG
SG_5LOXP_F2	TTGCAACACATTGATGAGCA
SG_5LOXP_R	CTTCCCCACAACGGGTTCTT
SG_3LOXP_F	GCCAGAGGCCACTTGTGTAG
SG_3LOXP_R2	TCGTTGCAACAAATTGATAAGC

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

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	Puro_3	GAGGCCTTCCATCTGTTGCT	GCGGTGTTCGCCGAGAT	CCGGGAACCGCTCAAC

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	-

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 wild type 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	Adnp_WT	GTTGACCTGTTGAGGCACAGT	ACAAGTGCTCAAATCCTAAGGA	CTGAGTGTTTGTCATCTCTT

Reaction setup

Reaction setup and amplification conditions are the same as those used for the neo cassette qPCR assay.

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. Mamm. Genome, 24, 286–294.

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. doi:10.1007/s11248-013-9764-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.