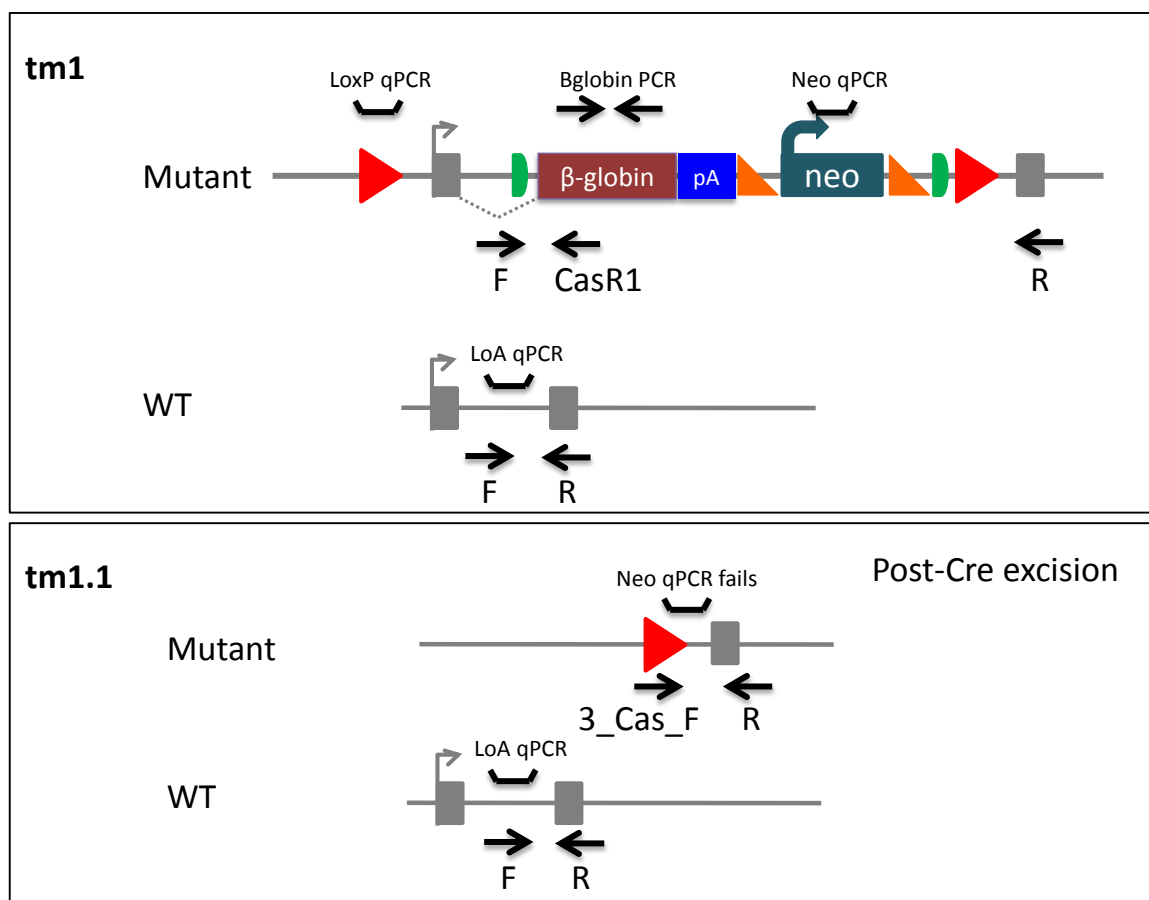**Gene**: 2810001G20Rik

Colony prefix: TABY

ESC clone ID: EPD01025_4_B12

Allele: *2810001G20Rik$^{tm1\ (NCC)WCS}$*

Allele type: non-coding RNA, Truncation cassette with conditional potential (selection cassette)

Allele information: http://www.mousephenotype.org/data/alleles/MGI:1913706/tm1(NCC)WCS?

Mouse QC information

Loss of WT Allele (LOA qPCR)	Pass	Neo qPCR	Pass
Mutant Specific SR-PCR	Pass	LoxP qPCR	Pass
Bglobin cassette SR-PCR	Pass		

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Genotyping by end-point PCR

PCRs primer pairs and expected size bands

Assay Type	Assay	Forward Primer	Reverse Primer	Expected Size Band
Standard PCR	Wild type	2810001G20Rik_WT_F	2810001G20Rik_WT_R	233
Standard PCR	Mutant	3_Cas_F	2810001G20Rik_R2	174
Standard PCR	Cassette	R-BGlobin_F	R-BGlobin_R	267

Primer sequences

Primer Name	Primer Sequence (5' > 3')
3_Cas_F	TCTATAGTCGCAGTAGGCGG
2810001G20Rik_WT_F	GCTTTGAAGAGCCAACTTGC
R-BGlobin_F	TGTTATATGGAGGGGGCAAA
2810001G20Rik_WT_R	AAGTGCTCGAAAAACAGTGGA
2810001G20Rik_R2	CTCCCCTAAGCAGCACTCAC
R-BGlobin_R	ACCCTGATTGCCTTGAAAAA

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	1:30 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	Neo	GGTGGAGAGGCTATTCGGC	GAACACGGCGGCATCAG	TGGGCACAACAGACAATCGGCTG

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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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

Gene	Forward Primer Seq.	Reverse Primer Seq.	Probe Primer Seq.	Source
2810001G20Rik	AGGCCCATCACAGTTCATC	TCACCAGAAATCCATCCTCTTC	TGTGGACCTAGGATAATGGTTGTCCC	Life Technologies

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-SNPTM kit (Applied Biosystems) from mouse ear biopsies. GTXpressTM buffer is also used (Applied Biosystems).

Reagent	µl
2x GTXpressTM	5
20x target assay	0.5
ddH2O	3
Tfrc endogenous 20x	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	-

Links to information and frequently asked questions

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

How the "critical" exon is decided:
http://www.i-dcc.org/kb/entry/102/

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.

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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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Farley, F.W., Soriano, P., Steffen, L.S., and Dymecki, S.M. (2000). Widespread recombinase expression using FLPeR (flipper) mice. Genesis 28, 106–110.