

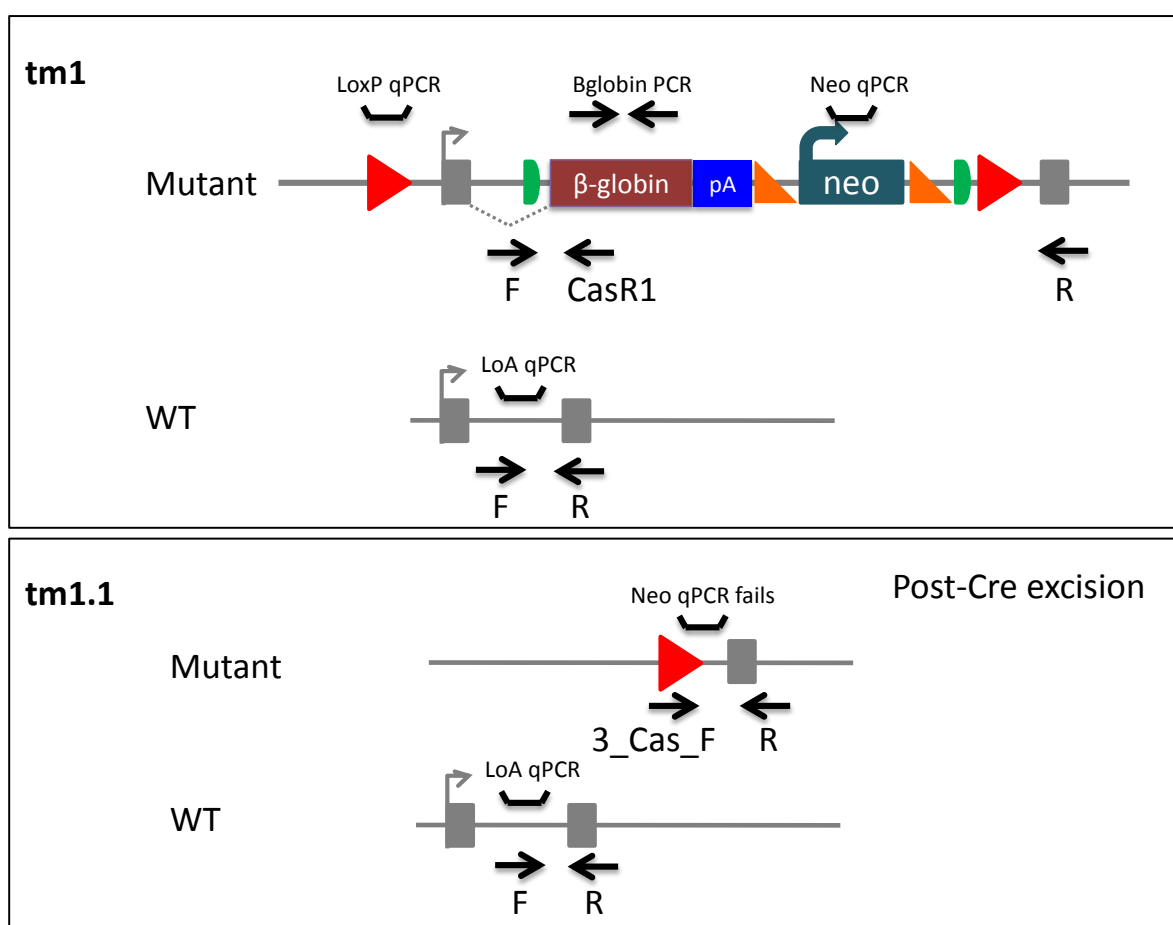
**Gene:** Cpgi23001

**Colony prefix:** TCAZ

**Allele:** *Cpgi23001*<sup>tm1.1(NCC)WCS</sup>

**Allele type:** non-coding RNA, post-Cre conversion

**Allele information:** [http://www.mousephenotype.org/data/alleles/MGI:5688069/tm1.1\(NCC\)WCS?](http://www.mousephenotype.org/data/alleles/MGI:5688069/tm1.1(NCC)WCS?)



### Mouse QC information

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

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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 (bp)
Standard PCR	Wild type	cpgi23001_1000341_F	cpgi23001_1000341_R	249
Standard PCR	Mutant	3_Cas_F	cpgi23001_1000341_R2	244

### Primer sequences

Primer Name	Primer Sequence (5' > 3')
cpgi23001_1000341_F	GCATGCTTGGTCCCTAAAAT
cpgi23001_1000341_R	TCCAGTTTTGTGCCCTACCT
cpgi23001_1000341_R2	GTCATGCACCACCATCAAAG
3_Cas_F	TCTATAGTCGCAGTAGGCGG

### Reaction setup

Reagent	µl
DNA (~50-100 ng)	1
10x Buffer	2
MgCl <sub>2</sub> (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
ddH <sub>2</sub> O	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 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
Cpgi23001	TTGTACCTCCAACCACATTAGG	TTGTCTGTCTCTGACCCTCT	AGGAAAGAGTTAGGTTCA GTGT GGA	Life Technologies

Reactions are performed in a 10µl volume using an Applied Biosystems 7900HT Fast Real-Time PCR System or Applied Biosystems Vii7 with DNA prepared using the Sample-to-SNPTM kit (Applied Biosystems) from mouse ear biopsies. GTXpress™ buffer is also used (Applied Biosystems).

Reagent	µl
2x GTXpress™ buffer	5
20x target assay	0.5
ddH <sub>2</sub> O	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	-

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## 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

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., Héroult, Y., and Pavlovic, G. (2011). Highly-efficient, fluorescent, locus directed Cre and flopo 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 FLP<sub>ER</sub> (flipper) mice. *Genesis* 28, 106–110.

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