

**Gene:** Cpgi15342

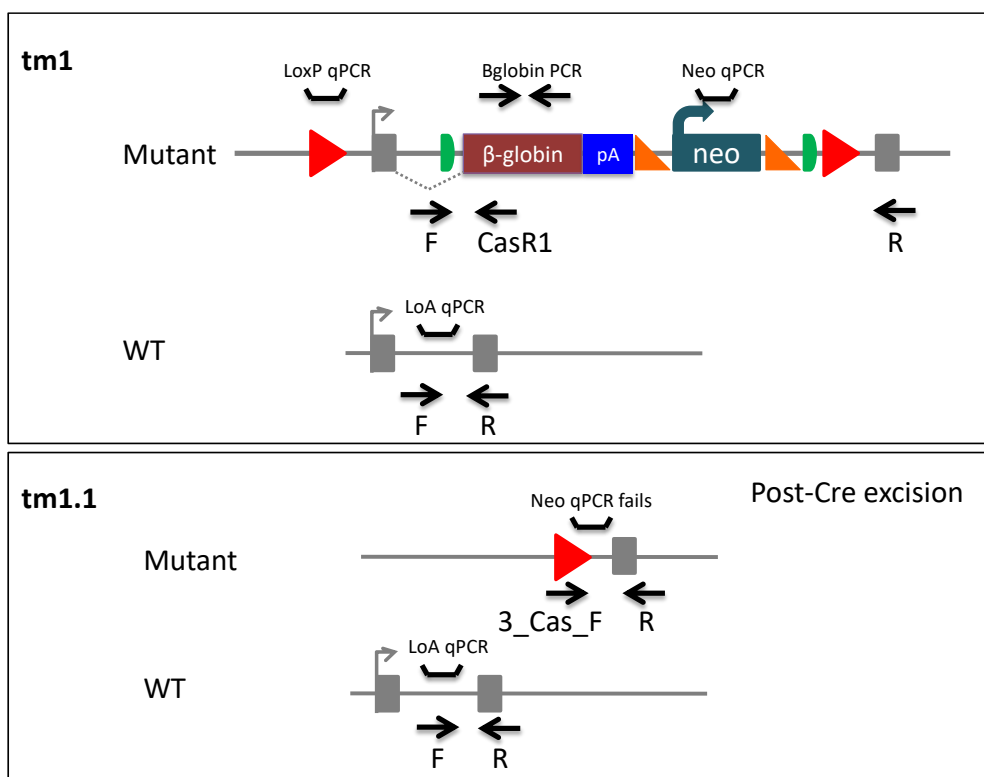
**Colony prefix:** TADU

**ESC clone ID:** EPD01017\_1\_B05

**Allele:** *Cpgi15342*<sup>tm1(NCC)WCS</sup>

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

**Allele information:** <http://www.mousephenotype.org>



#### Mouse QC information

|                                     |      |                  |      |
|-------------------------------------|------|------------------|------|
| <b>Loss of WT Allele (LOA qPCR)</b> | Pass | <b>Neo qPCR</b>  | Pass |
| <b>Mutant Specific SR-PCR</b>       | Pass | <b>LoxP qPCR</b> | Pass |
| <b>Bglobin cassette SR-PCR</b>      | 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 (bp) |
|--------------|-----------|---------------------|---------------------|-------------------------|
| Standard PCR | Wild type | Cpgi15342_1000378_F | Cpgi15342_1000378_R | 366                     |
| Standard PCR | Mutant    | 3_Cas_F             | Cpgi15342_1000378_R | 235                     |
| Standard PCR | Cassette  | R-BGlobin_F         | R-BGlobin_R         | 267                     |

### Primer sequences

| Primer Name         | Primer Sequence (5' > 3') |
|---------------------|---------------------------|
| 3_Cas_F             | TCTATAGTCGCAGTAGGCCGG     |
| Cpgi15342_1000378_F | TGAGACTGCAGCTTGGAAGA      |
| Cpgi15342_1000378_R | AGGCTTCCCTCTCTGTTTCC      |
| R-BGlobin_F         | TGTTATATGGAGGGGGCAA       |
| R-BGlobin_R         | ACCCTGATTGCCTTGAAAA       |

### 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 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.

| Assay Name | Forward Primer Seq. | Reverse Primer Seq. | Probe Primer Seq.       |
|------------|---------------------|---------------------|-------------------------|
| 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™ 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 |
| 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, Tfr; Applied Biosystems part #4458366). Reference DNA controls of known genotypes should also be included to facilitate correct analysis.

### Primers for LoA qPCR assay

| Assay Name   | Forward Primer Seq.     | Reverse Primer Seq.   | Probe Primer Seq.  |
|--------------|-------------------------|-----------------------|--------------------|
| Cpgi15342_WT | CCATTGGCAGAAGGTTGAAGTTG | CCACATTCCTGGATTGCTTCC | ATGTGATGTCCAGCTTCT |

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 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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## 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 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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