

Gene: Nubpl

Colony prefix: MMAX

ESC clone ID: EPD0216_2_C03

Allele: *Nubpl*^{tm1c(KOMP)Wtsi}

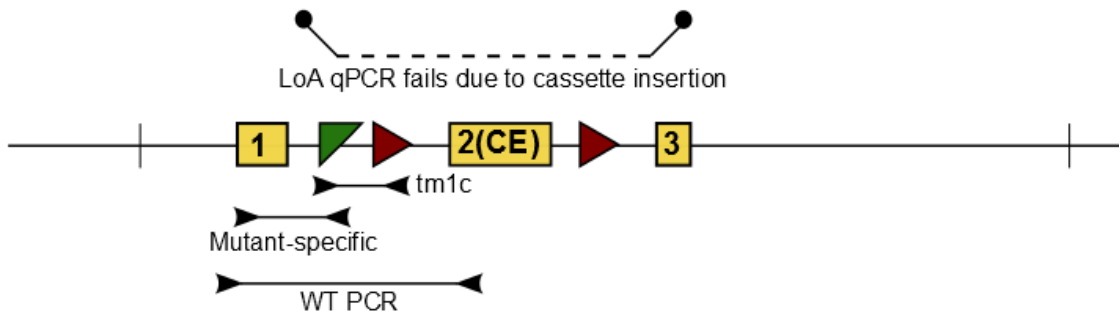
Allele type: *Conditional allele (post-Flp)*

Allele information:

Further information about the allele can be found on the IKMC web site at [http://www.mousephenotype.org/data/alleles/MGI:1924076/tm1c\(KOMP\)Wtsi](http://www.mousephenotype.org/data/alleles/MGI:1924076/tm1c(KOMP)Wtsi)? . Details on how to determine the floxed exon can be found at <http://www.knockoutmouse.org/kb/entry/21/>

Mouse QC information

Mutant allele



WT allele



| | | | | | |
|-------------------------------------|------|---|------|-------------------------|-----|
| Southern Blot | na | TV Backbone Assay | N/A | 5' LR-PCR | N/A |
| Loss of WT Allele (LOA) qPCR | pass | Homozygous Loss of WT Allele (LOA) SR- | pass | Neo Count (qPCR) | N/A |
| LacZ SR-PCR | N/A | 5' Cassette Integrity | N/A | Neo SR-PCR | N/A |
| Mutant Specific SR-PCR | pass | LoxP Confirmation | N/A | 3' LR-PCR | N/A |
| Genotyping Comment | | | | | |

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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/69506>

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.

Please note that for tm1c mice the mutant allele will still amplify a band in the WT assay, but the size will be different to the native WT product (exact size difference is allele-specific).

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PCRs primer pairs and expected size bands

| Assay Type | Assay | Forward Primer | Reverse Primer | Expected Size Band (bp) |
|--------------|----------|----------------|----------------|---------------------------|
| Standard PCR | Wildtype | Nubpl_F | Nubpl_R | . tm1c converted WT = 340 |
| Standard PCR | Mutant | Nubpl_F | CAS_R1_Term | 152 |
| Standard PCR | Cassette | Tm1c_F | Tm1c_R | 218 |

Primer sequences

| Primer Name | Primer Sequence (5' > 3') |
|-------------|---------------------------|
| CAS_R1_Term | TCGTGGTATCGTTATGCGCC |
| Tm1c_F | AAGGCGCATAACGATACCAC |
| Tm1c_R | CCGCCTACTGCGACTATAGAGA |
| Nubpl_F | TTCCATTCTTGACTAAGTTGCTG |
| Nubpl_R | ATAAATGCCCAACAAAACCCC |

Reaction setup

| Reagent | µl |
|---------------------------|-----------|
| DNA (~50-100 ng) | 1 |
| 10x Buffer | 2 |
| MgCl ₂ (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 ₂ 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 | 45 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

| Primer type | Assay Name | Forward Primer Seq. | Reverse Primer Seq. | Probe Primer Seq. |
|-------------|------------|-------------------------|-------------------------|-------------------|
| LoA | NUBPL_WT | CATGCAAGGCTGGCCTCTATTAT | TTCTGCTGTGTATCTGGCAAGAG | ATGTGCCGGTAGCCTGT |

Reaction setup

Reactions are performed in a 10µl volume using an Applied Biosystems 7900HT Fast Real-Time PCR System or Applied Biosystems Viiia7 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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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., Koutourakis, 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_{eR} (flipper) mice. *Genesis* 28, 106–110.

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