Genotyping protocol for Gnptab<nym>/+ mice. (RQ654).

Information taken from Emma submission form, private correspondence and Publication entitled.

A Novel Mouse Model of a Patient Mucolipidosis II Mutation Recapitulates Disease Pathology*
Nolan L et al, THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 289, NO. 39, pp. 26709–26721, September 26, 2014

The nym/nym mouse was identified from a phenotype-driven screen of the progeny from Balb/cAnNHsd N-ethyl-N-nitrosourea (ENU) mutagenized mice crossed to female C3H/HeNHsd engineered at MRC Harwell. The colony was maintained by back-crossing against C3H/HeNHsd.

Genetic description
Heterozygous mouse (Gnptabnym/+) carrying a truncation mutation in the Gnptab gene. This mutation introduces a T to A substitution at nucleotide 2601 of the cDNA sequence (T2601A) that changes the tyrosine into a premature stop codon at position 867 of the protein sequence (Y867X). Homozygous offspring (Gnptabnym/nym) by crossing heterozygous mice are mouse models of the human disease Mucolipidosis II.(The genetic background from the submission form is C3H/HeH (15 generations of backcrossing.)

PCR and digest.
Genotyping for the presence of the nym mutation was carried out using the primers
nym forward (5_-GGAGACGGTGACATACAAAAATCT-3_) and
nym reverse (5_-CACTGGATGCTCTAAGGAAGATAT-3_)
and subsequent digest with MseI, because this can cleave when the mutation is present.

The PCR product size was approximately 500 bp and the digest yielded 2 products of equal size (250bp) in mutant mice.

WT mice, single band at approx. 500bp.

Het mice gave a band at 500bp (WT) and at 250bp (mut).

Hom mice gave one band at 250 bp. (Providing the digestion was complete).

AR Feb 2015