**Genotyping protocol for Slc13a5-FLAG**

**There are two mutant sites in this line**

1. Neo cassette segment in the intron region
2. FLAG insertion:

GRCm38 (mm10): NC\_000077.6:g.72243505\_72243507delinsGGTTAATTAAGCCTGCTTGGCCTACTTGTCGTCATCGTCTTTGTAGTC;

NM\_001004148.4:c.1717\_1719delinsGACTACAAAGACGATGACGACAAGTAGGCCAAGCAGGCTTAATTAACC

Use this single primer set to amplify both WT and MU alleles

Slc13a5-OzFLAG-F: TCTGAAGTCACTTTCCAACATCA

Slc13a5-OzFLAG-R: CTTGTGCTCTTGCGGCTCT; RC: AGAGCCGCAAGAGCACAAG

The WT allele=339 bp

The MU allele=460 bp

Sanger sequencing revealed that there was a Neomycin fragment in Intron 11 on the FLAG allele beside the 1X FLAG sequence, which resulted in a 121 bp size difference between the WT allele and the FLAG allele in Intron 11 and Exon 12. This size difference allows analysis on a 2% agarose gel. A single primer set was designed to amply both alleles and run on a 2% agarose gel.

Each PCR reaction contained 10 µL of Platinum Hot Start PCR Master Mix (2x) (Invitrogen, Carlsbad, CA, USA), 1 µL of 10 µM primer mix, 2 µL of DNA template and raised to 20 µL with distilled water. The reactions were run using a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA) Thermocycler.

PCR conditions: initial denaturation @ 94 °C for 2 min, then [30 cycles of 94 °C for 30 s (template denaturation) then 59 °C for 30 s (primer annealing) followed by 72 °C for 30 s (primer extension)], 72 °C for 2 min and then hold at 4 °C.

>*Slc13a5*-WT

TCTGAAGTCACTTTCCAACATCACAATGGGTGTAGAGAACCCACCGTGCTGGCTCCTTCCAAGGGATTCTATCAGCGACAATGACACTTAATTTGCTTTGTCCTGAGACGAGAGGAGCCCAGTGTGCAGGAACACAAGGCCCAGTCAAGACCTGAGTTGCTGACTGATCCTGTTTTTACTCCCTAGATGAAAACAGGATTGATAATGAACTTCGTTGGAATCCTATCTGTGTTTCTGTCAGTCAACACCTGGGGTCGGGCTATGTTTAACTTGGATAACTTCCCCGACTGGGCAAATTCAACAAGTGTTAACACTTAGGAAGAGCCGCAAGAGCACAAG

>Slc13a5-FLAG

TCTGAAGTCACTTTCCAACATCACAATGGGATAGGCGCGCCGAAGTTCCTATTCCGAAGTTCCTATTCTCTAGTAAGTATAGGAACTTCTTATGATCTAAGGTACCTGTAGAGAACCCACCGTGCTGGCTCCTTCCAAGGGATTCTATCAGCGACAATGACACTTAATTTGCTTTGTCCTGAGACGAGAGGAGCCCAGTGTGCAGGAACACAAGGCCCAGTCAAGACCTGAGTTGCTGACTGATCCTGTTTTTACTCCCTAGATGAAAACAGGATTGATAATGAACTTCGTTGGAATCCTATCTGTGTTTCTGTCAGTCAACACCTGGGGTCGGGCTATGTTTAACTTGGATAACTTCCCCGACTGGGCAAATTCAACAAGTGTTAACACTGACTACAAAGACGATGACGACAAGTAGGCCAAGCAGGCTTAATTAACCGAAGAGCCGCAAGAGCACAAG



M: 1 Kb Plus DNA Ladder (Invitrogen, Carlsbad, CA, USA)