Gene: Slc13a5 ENSMUSG00000020805; Indy, NaC2/NaCT, Nact, mINDY

**Genotyping protocol for Slc13a5-19del**

NM\_001004148.4:c.1685\_1703delCCGACTGGGCAAATTCAAC

First, perform PCR using the following primer set

Slc13a5-406-F: ACAAAATGGGTGGCAGAAAG

Slc13a5-427-R: CCAGCATGGAGCCAGTAGTC; RC: GACTACTGGCTCCATGCTGG

Amplicon size:

WT= 719 bp

MU (Slc13a5-19del)= 700 bp

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 (final conc. <500 ng/rxn) 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.

The reaction conditions were: template 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 60 s (primer extension)], 72 °C for 2 min and then hold at 4 °C.

>*Slc13a5*-WT

ACAAAATGGGTGGCAGAAAGAGCTATGGGAGAGTCACGAGACGTGGCTGATGTCTTACACCAGCTCTCTGAAGTCACTTTCCAACATCACAATGGGTGTAGAGAACCCACCGTGCTGGCTCCTTCCAAGGGATTCTATCAGCGACAATGACACTTMluCIAATTTGCTTTGTCCTGAGACGAGAGGAGCCCAGTGTGCAGGAACACAAGGCCCAGTCAAGACCTGAGTTGCTGACTGATCCTGTTTTTACTCCCTAGATGAAAACAGGATTGATAATGAACTTCGTTGGAATCCTATCTGTGTTTCTGTCAGTCAACACCTGGGGTCGGGCTATGTTTAACTTGGATAACTTCCCCGACTGGGCAMluCIAATTCAACAAGTGTTAACACTTAGGAAGAGCCGCAAGAGCACAAGCATGCCCCCCCCACCCCAACCCTTTTGAGGACTATGAACCTTCTGGCACACCTTGCACAGAGCACTGGTGCTCACACTCTAGTGTGATCCAATGATGTCAACACCCCAAGAAGATCTACCCAACTATATCAAGTTCAGAGATGGCAATGGATGATGGGAAGATAAGCTCAGAAGGGAAAGGAACCCCTTTGAGAGGTCGCGAGGCCCATCTTTACTAGGACCCTTCCATCTCTCCTGGGACAGGCAGGAACCAGAGGGACCAGGGCTCAAATCTTGTACTATGTGGCTTTGACAGACTACTGGCTCCATGCTGG

>*Slc13a5*-19del

ACAAAATGGGTGGCAGAAAGAGCTATGGGAGAGTCACGAGACGTGGCTGATGTCTTACACCAGCTCTCTGAAGTCACTTTCCAACATCACAATGGGTGTAGAGAACCCACCGTGCTGGCTCCTTCCAAGGGATTCTATCAGCGACAATGACACTTMluCIAATTTGCTTTGTCCTGAGACGAGAGGAGCCCAGTGTGCAGGAACACAAGGCCCAGTCAAGACCTGAGTTGCTGACTGATCCTGTTTTTACTCCCTAGATGAAAACAGGATTGATAATGAACTTCGTTGGAATCCTATCTGTGTTTCTGTCAGTCAACACCTGGGGTCGGGCTATGTTTAACTTGGATAACTTCCAAGTGTTAACACTTAGGAAGAGCCGCAAGAGCACAAGCATGCCCCCCCCACCCCAACCCTTTTGAGGACTATGAACCTTCTGGCACACCTTGCACAGAGCACTGGTGCTCACACTCTAGTGTGATCCAATGATGTCAACACCCCAAGAAGATCTACCCAACTATATCAAGTTCAGAGATGGCAATGGATGATGGGAAGATAAGCTCAGAAGGGAAAGGAACCCCTTTGAGAGGTCGCGAGGCCCATCTTTACTAGGACCCTTCCATCTCTCCTGGGACAGGCAGGAACCAGAGGGACCAGGGCTCAAATCTTGTACTATGTGGCTTTGACAGACTACTGGCTCCATGCTGG

Then, perform enzyme digestion with enzyme digestion with MluCI (NEB, Ipswich, MA, USA)

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| MluCI Enzyme 0.6 ul10xNEBuffer (CutSmart) 2.5 ulPCR product 10 ulddH2O 11.9 ulTotal 25 ul |
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Incubate for 30 mins at 37⁰C at a GeneAmp PCR System 9700 Thermocycler

The WT and MU alleles were cut into the following fragments

WT= 155, 205 and 359 bp

MU (19 del)= 155 and 545 bp



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