NAME: Eric Delpire

DATE: 7/04/18

VRSN: 01

GENE: Slc12a2

MOUS: B6-Slc12a2tm2Del

MUTA: Val1026Phefs∗2

PUBL: Koumangoye R., Omer S., and E. Delpire. Mistargeting of a truncated Na-K-2Cl cotransporter in epithelial cells. *Am. J. Physiol. Cell Physiol.* In Press, 2018.

Delpire E., Wolfe L., Flores B., Koumangoye R., Schornak C.C., Omer S., Pusey B., Lau C., Markello T., and D.R. Adams. A patient with multisystem dysfunction carries a truncation mutation in human SLC12A2, the gene encoding the Na-K-2Cl cotransporter, NKCC1. *Cold Spring Harb. Mol. Case Studies* 2: a001289, 2016.

DESC: Using CRISPR, 11 bp were deleted within exon 22 of Slc12a2. In addition, with the addition of an A>C mutation in the codon that encodes Isoleucine (ATA > ATC), a ClaI site appears. Out of 20 pups produced and genotyped, one was identified with the 11 bp deletion in one allele and a non-end joining mutation in the other allele. The mouse was a functional knockout. The alleles were separated by crossing with C57BL/6J male and the NKCC1-DFX line was backcrossed for >7 generations in C57BL6/J.

WILD-TYPE GENOMIC FRAGMENT:

001..027 Forward primer: AAGCTCCTTCAGACTTAAGATGGAAGC

150..272 exon 2 (123 bp)

Translation: “SKGPIVPLNVADQKLLEASTQFQKKQGKNTIDVWWLFDDG”

246..268 GGAAGAATACTATAGATGTC TGG gRNA (positive strand)

395..421 Reverse primer: ACCTACAGCTCTCATGGTGATTCTTAC

1. AAGCTCCTTC AGACTTAAGA TGGAAGCCAT ATACAGCTAT CTGCATATCA GTATCTGAAT

61. TTACAAATGC TATGAAATTC TAGGAATATG GTGAAAAGAG ATTTCACATT TACTTCCCTT

121. ATGTCATAAT TTTTTGCTTA ATTCTTCAGA ATCCAAAGGC CCTATTGTGC CTTTAAATGT

181. AGCTGACCAA AAGCTTCTTG AAGCTAGCAC ACAGTTTCAG AAAAAACAAG GGAAGAATAC

241. TATAGATGTC TGGTGGCTTT TTGATGATGG AGGTAAGGCC ATTAATGTAT TTATTTTATA

301. AAGAATTACT GAAAATGTAA GAATAGTGCT AAGAAATGGC ATGCTTTCTT CCTGTGTTTA

361. CCCCGTTCAG GTCTCACTAG CATCCTCAAA CAGGGTAAGA ATCACCATGA GAGCTGTAGG

421. T

MUTANT GENOMIC FRAGMENT:

001..027 Forward primer: AAGCTCCTTCAGACTTAAGATGGAAGC

150..261 exon 2 (112 bp)

Translation: “SKGPIVPLNVADQKLLEASTQFQKKQGKNTIDF\*”

124..286 163 base repair oligonucleotide (forward strand)

242..247 ClaI site introduced

397..420 Reverse primer: ACCTACAGCTCTCATGGTGATTCTTAC

1. AAGCTCCTTC AGACTTAAGA TGGAAGCCAT ATACAGCTAT CTGCATATCA GTATCTGAAT

61. TTACAAATGC TATGAAATTC TAGGAATATG GTGAAAAGAG ATTTCACATT TACTTCCCTT

121. ATGTCATAAT TTTTTGCTTA ATTCTTCAGA ATCCAAAGGC CCTATTGTGC CTTTAAATGT

181. AGCTGACCAA AAGCTTCTTG AAGCTAGCAC ACAGTTTCAG AAAAAACAAG GGAAGAATAC

241. TATcGATTTT TGATGATGGA GGTAAGGCCA TTAATGTATT TATTTTATAA AGAATTACTG

301. AAAATGTAAG AATAGTGCTA AGAAATGGCA TGCTTTCTTC CTGTGTTTAC CCCGTTCAGG

361. TCTCACTAGC ATCCTCAAAC AGGGTAAGAA TCACCATGAG AGCTGTAGGT

