NAME: Eric Delpire

DATE: 8/05/15

VRSN: 01

GENE: Slc12a5

K-Cl cotransporter 2 (KCC2)

MGI#: 1862037

CHR#: 2 [164.9-165.0 Mb]

MOUS: B6.Slc12a5tm1Del

DESC: Using homologous recombination in ES cells, exon 1b was targeted to introduce a neomycin-resistance gene cassette, thereby preventing the use of exon 1b and the production of a KCC2-1b protein. A 1714 bp XhoI – BamHI fragment was deleted and replaced by the neomycin-resistance gene cassette.

RESD: NheI + BamHI (3000 bp for wild-type and 4500 bp for mutant)

PROB: NheI-SphI fragment at 5’ end

gctagcttcaaacttgtcttgagagccctgcttgcaactcctaataggtgtgcacccctaccctcagctaggtacccccaaatacttttgtggtattggggattgaacatggagccttgtgcatattagacaagctctcacccacagagctatggtcttaatctaagactcatagaagacaggaaggaatctctcctttattttatggatgcagatgctgaagaccagagagagggactagattttgaccaggaataagcagaattctgcagcagaggagaggatagaacccgtatttcccacatcttgagtggccttccaggtcagagctctagtgaggattggcagtgaggcagtcactctggccttcatctgtttctcacgtccccacggataggtttactcccagggtgatcagagaagcaatggcaccaggaccagagatgaagaagcatgc

CLON: 1B6, 1F8, 1F12, 2B9, 2F5

GERM: 1F12 and 2B9, October 18, 1999

BCKG: >10 generations in C57BL/6J.

NOTE: The mouse slc12a5 gene is located on chromosome 2 not too far from the agouti coat color gene (10 cM).

NOTE: Wild-type allele is identified with primers FP1 – RP1

Mutant allele is identified with primers FP1 – RP2

FP1: AGCGTGTGTCCGTGTGCGAGTG within exon 1b

RP1: CCGTCCTCGCAGTCCGTCAGGTTG within following intron

RP2: CCAGAGGCCACTTGTGTAGCGC within PGK-1

CONSTRUCT:

1..2249 left (short) arm of recombination (SphI – XhoI fragment)

1912..2249 exon 1, 5’UTR

2157..2178 Forward Primer 1

2269..2278 PGK-1 promoter

2339..2360 Reverse Primer 2(RP2)

2783..3583 neomycin

\translation=”mgsaieqdglhagspaawverlfgydwaqqtigcsdaavfrlsaqgrpvlfvktdlsgaL nelqdeaarlswlattgvpcaavldvvteagrdwlllgevpgqdllsshlapaekvsimadamrrlhtldpatcpfdhqakhrierartrmeaglvdqddldeehqglapaelfarlkarmpdgedlvvthgdaclpnimvengrfsgfidcgrlgvadryqdialatrdiaeelggewadrflvlygiaapdsqriafyrlldeff“

3597..4069 pA

4076..10784 right (long) arm of recombination (6.7 kb BamHI fragment)

7287..7381 exon 2 (55 bp)

8048..8179 exon 3 (132 bp)

8507..8657 exon 4 (147 bp)

9484..9538 exon 5 (55 bp)

10033..10163 exon 6 (131 bp)

18806..11315 PGK-1 promoter

11539..12531 thymidine kinase

\translation=”mptllrvyidgphgmgkttttqllvalgsrddivyvpepmtywqvlgasetianiyttqh rldqgeisagdaavvmtsaqitmgmpyvtdavlaphiggeagsshapppaltlifdrhpiaallcypaarylmgsmtpqavlafvalipptlpgtnivlgalpedrhidrlakrqrpgerldlamlaairrvygllantvrylqgggswredwgqlsgtavppqgaepqsnagprphigdtlftlfrapellapngdlynvfawaldvlakrlrpmhvfildydqspagyrdallqltsgmvqthvttpgsipticdlartfaremgean”

13142..13593 pA

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