

NAME: Eric Delpire

DATE: 7/21/18

VRSN: 01

GENE: DNPEP

MOUS: B6.B6D2F1-Dnpep^{em1Del}

MUTA: K21X

PUBL: Koumangoye, R. and E. Delpire. DNPEP is not the only peptidase that produces SPAK fragments in kidney. *Physiol Rep* 5: pii: e13479, 2017.

DESC: Using CRISPR, the codon encoding Lys21 in Dnpep, exon 2, was modified to encode a stop codon. Additional silent mutations were introduced to 1) create a BspHI restriction site for genotyping purposes; and 2) protect the repaired DNA from sgRNA-mediated cas9 targeting. Out of 31 pups produced and genotyped, 5 pups were identified with the AAG (Lys) > TAG (stop codon) mutation, all of them had 100% matching sequences. Line 17 was backcrossed for 8 generations in C57BL6/J.

WILD-TYPE GENOMIC FRAGMENT:

001..022 Forward primer: GACAAAACTGGGTGGAGGTCCTC
194..287 exon 2 (94 bp)
Translation: "MAMNGRARKEAIQATARELLKFVNRSPPFH"
246..268 AGCTCCTGAAGTTCGTGAAC CCG gRNA (positive strand)
397..420 Reverse primer: CTTGAGTTCACGGAAGCCAGCCTG

1. GACAAAACTG GGTGGAGGTC CTCTTGCCCT GGGACTGAAC CTTTGAGCCG AGAGCGATCG
61. AGCTAGGAAT CCACATCTGT CGCCAGTGAA GTCCTCGGAA GCAGAGGTTG AGCCTTGGGG
121. CTCTCATTTT TAGGGGGCTG AAAGAGGGAT CCTAGACCCG GCAGCCACCT GAACTCTCCA
181. CTTCTCCCTG TAGATGGCTA TGAACGGCAG GGCTCGGAAA GAGGCCATCC AGGCCACAGC
241. CCGAGAGCTC CTGAAGTTCG TGAACCGGAG TCCCTCTCCT TTCCACGGTA GGTGACCAGG
300. GTTAGGGAGG CTAGGAGTGG GAGTGACTTG ACCAACTTCC ATCGCTGAGA GAGCCCTTTT
360. CCTACAGTCG TGGCTGAGTG CCGCAGCCGC CTCCTCCAGG CTGGCTTCCG TGAACTCAAG

MUTANT GENOMIC FRAGMENT:

001..022 Forward primer: AGAACCTTCGGCAGAAGTGAGTC
194..287 exon 2 (94 bp)
Translation: "MAMNGRARKEAIQATARELL*S*NRSPSPFH"
166..356 191 base repair oligonucleotide (forward strand)
257..262 BspHI site introduced
397..420 Reverse primer: CTTGAGTTCACGGAAGCCAGCCTG

1. GACAAAACTG GGTGGAGGTC CTCTTGCCCT GGGACTGAAC CTTTGAGCCG AGAGCGATCG
61. AGCTAGGAAT CCACATCTGT CGCCAGTGAA GTCCTCGGAA GCAGAGGTTG AGCCTTGGGG
121. CTCTCATTTT TAGGGGGCTG AAAGAGGGAT CCTAGACCCG GCAGCCACCT GAACTCTCCA
181. CTTCTCCCTG TAGATGGCTA TGAACGGCAG GGCTCGGAAA GAGGCCATCC AGGCCACAGC
241. CCGAGAGCTC TCCCTCTCCT CTGTAGTCAT GAAATCGAAG TTCCACGGTA GGTGACCAGG
300. GTTAGGGAGG CTAGGAGTGG GAGTGACTTG ACCAACTTCC ATCGCTGAGA GAGCCCTTTT
360. CCTACAGTCG TGGCTGAGTG CCGCAGCCGC CTCCTCCAGG CTGGCTTCCG TGAACTCAAG

