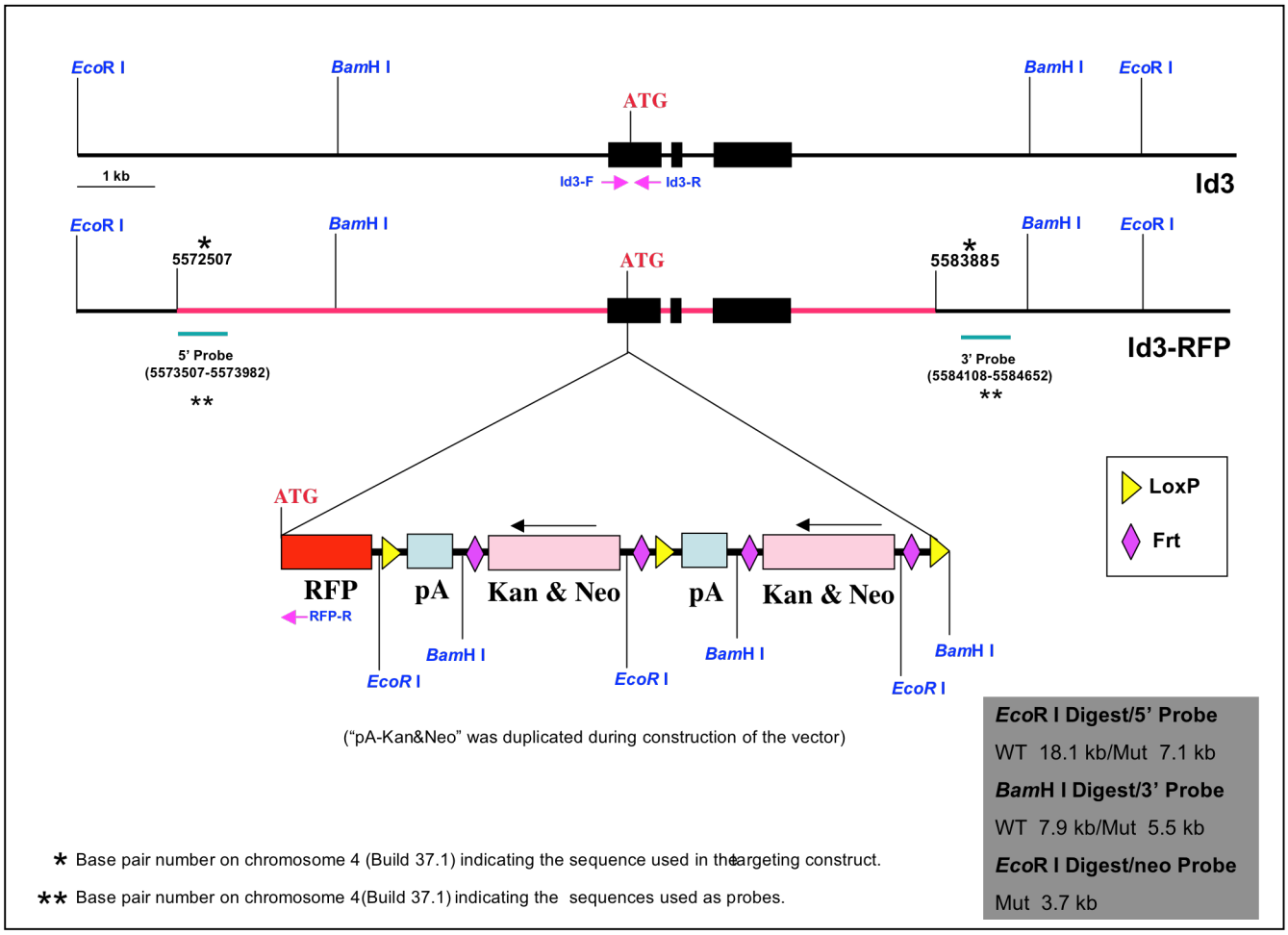
Id3

A. Rationale

Id3 expression is not confined to a known cell type within the developing metanephros. An Id3 knock-in approach was employed by the GUDMAP consortium as a means of investigating this population of cells in more detail.

B. Targeting Strategy



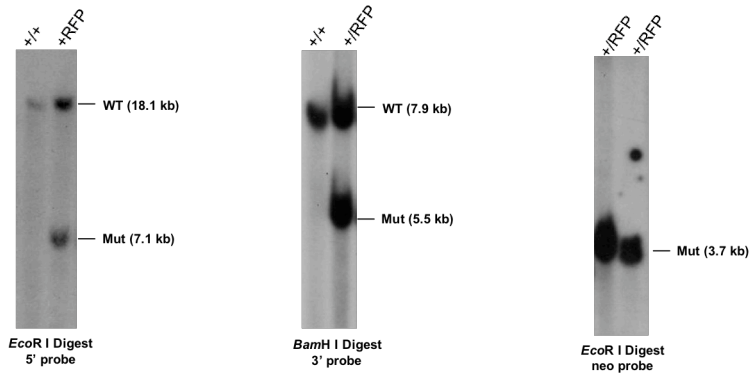
("pA-Kan&Neo" was duplicated during construction of the vector)

***Eco*R I Digest/5' Probe**
WT 18.1 kb/Mut 7.1 kb
***Bam*H I Digest/3' Probe**
WT 7.9 kb/Mut 5.5 kb
***Eco*R I Digest/neo Probe**
Mut 3.7 kb

* Base pair number on chromosome 4 (Build 37.1) indicating the sequence used in the targeting construct.

** Base pair number on chromosome 4 (Build 37.1) indicating the sequences used as probes.

C. Southern Blot Analysis of the Targeted Allele in Mice



Genomic DNA isolated from wildtype and heterozygous mice were digested with the indicated restriction enzymes, separated in 0.8% agarose gel, transferred to charged nylon membrane, and hybridized with ^{32}P-labeled probes. The autoradiograms are shown.

D. PCR Genotyping

a. Primers

Id3-F: 5’ tcctcggtatcagcgcttcc 3’
Id3-R: 5’ caatggccaggctacgttcc 3’
RFP-R: 5’ cttgatgacgtcctcggagg 3’

b. Expected Band Sizes

Id3-F + Id3-R: 351 bp
Id3-F + RFP-R: 234 bp

E. Relevant Sequences

a. Genomic clone used for targeting construct

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b. The final construct (exclude plasmid backbone and the negative selection marker)

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Id3-F

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taaggggtgtgtcctagagaggactctataagagtcggccgctgcaggcgtgcgcgcACTGTTTGCTGCTTTAGGTGTCT

Start of Id3 transcription

CTTTTCCTCCCTCTCTATCTCTACTCTCCAAC ATGGCCTCCTCCGAGGACGTCATCAAGGAGTTCATGCGCTTCAAGGTG

RFP-R

Start of RFP

CGCATGGAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCAGACC
GCCAAGCTGAAGGTGACCAAGGGCGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCCAGTACGGCTCCAAG
GCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATG
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CGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCACCGAGCGGATGTAC
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ACCACCTACATGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAAGACCGACATCAAGCTGGACATCACCTCCCACAAC
GAGGACTACACCATCGTGGAACAGTACGAGCGCGCCGAGGGCCGCCACTCCACCGGCGCCTAA gaattcctgcagcccaa

End of RFP

ttccgatcatattcaataacccttaat ataacttcgtataatgtatgctatacgaagttat CTGCAGGCGCGCCCCTAG

LoxP

Start of bGH polyA

GGCGGCCGCCACCTCGAGGGGGCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCC
CGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAG

TAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGG
GGATGCGGTGGGCTCGAGATCCACTAGTTCTAGCCTCGAGGCTAGAGCGGCCGCCAccgcggcgtagaggatctgttgatc

End of bGH polyA

agcagttcaacctgttgatagtacgtactaagctctcatgtttcacgtactaagctctcatgtttaacgtactaagctctc
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cagggcgcaagggctgctaaaggaagcggaacacgggatccatgcatagatccccccctcgaaaacgctagcgttaattaa
gaagttcctatacttttagagaataggaacttc AGCTTCTGATGGAATTAGAACTTGGCAAAACAATACTGAGAATGA

Frt End of Kan^{R}

AGTGTATGTGGAACAGAGGCTGCTGATCTCGTTCTTCAGGCTATGAAACTGACACATTTGGAAACCACAGTACTTAGAACA
CAAAGTGGGAATCAAGAGAAAAACAATGATCCCACGAGAGATCCATGCATAGATCTTAATTAATTAGAAAAACTCCATCGA
GCATCAAATGAAACTGCAATTTATTCATATCAGGATTATCAATACCATATTTTTGAAAAAGCCGTTTCTGTAATGAAGGAG
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Start of Kan^{R} End of bGH polyA

CCACCCCACCCCCCAGAATAGAATGACACCTACTCAGACAATGCGATGCAATTTCCTCATTTTATTAGGAAAGGACAGTGG
GAGTGGCACCTTCCAGGGTCAAGGAAGGCACGGGGGAGGGGCAAACAACAGATGGCTGGCAACTAGAAGGCACAGTCGAGG
CTGATCAGCGAGCTCTAGAGAATTGATCCCCCC TCAGAAGAACTCGTCAAGAAGGCGATAGAAGGCGATGCGCTGCGAAT

Start of bGH polyA End of Neo

CGGGAGCGGCGATACCGTAAAGCACGAGGAAGCGGTCAGCCCATTCGCCGCCAAGCTCTTCAGCAATATCACGGGTAGCCA
ACGCTATGTCCTGATAGCGGTCCGCCACACCCAGCCGGCCACAGTCGATGAATCCAGAAAAGCGGCCATTTTCCACCATGA
TATTCGGCAAGCAGGCATCGCCATGGGTCACGACGAGATCCTCGCCGTCGGGCATGCGCGCCTTGAGCCTGGCGAACAGTT
CGGCTGGCGCGAGCCCCTGATGCTCTTCGTCCAGATCATCCTGATCGACAAGACCGGCTTCCATCCGAGTACGTGCTCGCT
CGATGCGATGTTTCGCTTGGTGGTCGAATGGGCAGGTAGCCGGATCAAGCGTATGCAGCCGCCGCATTGCATCAGCCATGA
TGGATACTTTCTCGGCAGGAGCAAGGTGAGATGACAGGAGATCCTGCCCCGGCACTTCGCCCAATAGCAGCCAGTCCCTTC
CCGCTTCAGTGACAACGTCGAGCACAGCTGCGCAAGGAACGCCCGTCGTGGCCAGCCACGATAGCCGCGCTGCCTCGTCCT
GCAGTTCATTCAGGGCACCGGACAGGTCGGTCTTGACAAAAAGAACCGGGCGCCCCTGCGCTGACAGCCGGAACACGGCGG
CATCAGAGCAGCCGATTGTCTGTTGTGCCCAGTCATAGCCGAATAGCCTCTCCACCCAAGCGGCCGGAGAACCTGCGTGCA
ATCCATCTTGTTCAATGGCCGATCCCATATTGGCTGCAGGTCGAAAGGCCCGGAGATGAGGAAGAGGAGAACAGCGCGGCA
GACGTGCGCTTTTGAAGCGTGCGAGAATGCCGGGCCTCCGGAGGACCTTCGGGCGCCCGCCCCGCCCCTGAGCCCGCCCCT
GAGCCCGCCCCCGGACCCACCCCTTCCCAGCCTCTGAGCCCAGAAAGCGAAGGAGCAAAGCTGCTATTGGCCGCTGCCCCA
AAGGCCTACCCGCTTCCAT tgctcagcggtgctgtccatctgcacgagactagtgagacgtgctacttccatttgtcacg

Start of Neo End of PGK promoter

Tcctgcacgacgcgagctgcggggcgggggggaacttcctgactaggggaggagtagaaggtggcgcgaaggggccaccaa
agaacggagccggttggcgcctaccggtggatgtggaatgtgtgcgaggccagaggccacttgtgtagcgccaagtgccag
cggggctgctaaagcgcatgctccagactgccttgggaaaagcgcctcccctacccggtag aattcga gaagttcctat

Start of PGK promoter

acttttagagaataggaacttc gatcc ataacttcgtataatgtatgctatacgaagttat CTGCAGGCGCGCCCCT


Frt
LoxP
Start of bGH polyA
AGGGCGGCCGCCACCTCGAGGGGGCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCC
CCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTG
AGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCT
GGGGATGCGGTGGGCTCGAGATCCACTAGTTCTAGCCTCGAGGCTAGAGCGGCCGCCA ccgcggcgtagaggatctgttg
End of bGH polyA
atcagcagttcaacctgttgatagtacgtactaagctctcatgtttcacgtactaagctctcatgtttaacgtactaagct
ctcatgtttaacgaactaaaccctcatggctaacgtactaagctctcatggctaacgtactaagctctcatgtttcacgta
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aa gaagttcctatacttttagagaataggaacttc AGCTTCTGATGGAATTAGAACTTGGCAAAACAATACTGAGAAT
Frt
End of Kan^R
GAAGTGTATGTGGAACAGAGGCTGCTGATCTCGTTCTTCAGGCTATGAAACTGACACATTTGGAAACCACAGTACTTAGAA
CACAAAGTGGGAATCAAGAGAAAAACAATGATCCCACGAGAGATCCATGCATAGATCTTAATTAATTAGAAAAACTCCATC
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GCTAGAACTAGTGGATCTCGAGCCCACCGCATCCCCAGCATGCCTGCT ATTGTCTTCCCAATCCTCCCCCTTGCTGTCCT
Start of Kan^R
End of bGH polyA
GCCCCACCCCACCCCCCAGAATAGAATGACACCTACTCAGACAATGCGATGCAATTTCCTCATTTTATTAGGAAAGGACAG
TGGGAGTGGCACCTTCCAGGGTCAAGGAAGGCACGGGGGAGGGGCAAACAACAGATGGCTGGCAACTAGAAGGCACAGTCG
AGGCTGATCAGCGAGCTCTAGAGAATTGATCCCCCC TCAGAAGAACTCGTCAAGAAGGCGATAGAAGGCGATGCGCTGCG
Start of bGH polyA
End of Neo
AATCGGGAGCGGCGATACCGTAAAGCACGAGGAAGCGGTCAGCCCATTCGCCGCCAAGCTCTTCAGCAATATCACGGGTAG
CCAACGCTATGTCCTGATAGCGGTCCGCCACACCCAGCCGGCCACAGTCGATGAATCCAGAAAAGCGGCCATTTTCCACCA
TGATATTCGGCAAGCAGGCATCGCCATGGGTCACGACGAGATCCTCGCCGTCGGGCATGCGCGCCTTGAGCCTGGCGAACA
GTTCGGCTGGCGCGAGCCCCTGATGCTCTTCGTCCAGATCATCCTGATCGACAAGACCGGCTTCCATCCGAGTACGTGCTC
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CCTGCAGTTCATTCAGGGCACCGGACAGGTCGGTCTTGACAAAAAGAACCGGGCGCCCCTGCGCTGACAGCCGGAACACGG
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CCTGAGCCCGCCCCCGGACCCACCCCTTCCCAGCCTCTGAGCCCAGAAAGCGAAGGAGCAAAGCTGCTATTGGCCGCTGCC
CCAAAGGCCTACCCGCTTCCAT tgctcagcggtgctgtccatctgcacgagactagtgagacgtgctacttccatttgtc
Start of Neo
End of PGK promoter
acgtcctgcacgacgcgagctgcggggcgggggggaacttcctgactaggggaggagtagaaggtggcgcgaaggggccac
caaagaacggagccggttggcgcctaccggtggatgtggaatgtgtgcgaggccagaggccacttgtgtagcgccaagtgc
cagcggggctgctaaagcgcatgctccagactgccttgggaaaagcgcctcccctacccggtagaattcga gaagttcct
Start of PGK promoter

atacttttagagaataggaacttc gatcc ataacttcgtataatgtatgctatacgaagttat taggtccctcgagg

Frt LoxP

ggatccac AAGGCGCTGAGCCCGGTGCGCGGCTGCTACGAGGCGGTGTGCTGCCTGTCGGAACGTAGCCTGGCCATTGCG

Id3 continues Id3-R

CGAGGCCGCGGTAAGAGCCCGTCGACCGAGGAGCCTCTTAGCCTCTTGGACGACATGAACCACTGCTACTCGCGCCTGCGG
GAACTGGTGCCGGGAGTCCCGCGAGGCACTCAGCTTAGCCAGGTGGAAATCCTGCAGCGTGTCATAGACTACATCCTCGAC
CTTCAGGTGGTCCTGGCAGAGCCGGCGCCTGGACCCCCGGACGGTCCGCATCTCCCGATCCAGgtgcgagagggagccaga

Exon/intron junction

ccaggctgctctgagcgtgcgggcagggatgctgcgggtcttccctatcgcgtccccgagtcccttggctaactcgtctcc
taaccttctttcacagACAGCTGAGCTCACTCCGGAACTTGTGATCTCCAAGGACAAGAGGAGCTTTTGCCACTGACCCGG

Intron/exon junction

TCGTCCTGGCACCTCCCGgtaagctttctcctggcgcgggcgaggagggaggcttgcatgggaaatcctgcctttgacaga

Exon/intron junction

Acattgtaaggcttagggttcagtcggtttagggaaaaagccaagccactgaaaggcaaaagccttatctataatcagtta
gaataaacgacagaacctatgtcaatatcacgtgcattccttagacacgctgtcccttctcatcccgggtggccagagccc
agggcaagtgggcgcgcgggtcgcaccaaatgagccttggagtaaaggaagccctccccccttccactagtgttatttct
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acggctccctcgctcttctctcctcccccgcccagAACGCAGGTGCTGGCGCCCGTTCCGCTTGGGACCCTGGGACTCTGG

Intron/exon junction

GACCCTCTCTCCAGCCGGAAGCCTGAGGGCATGGATGAGCTTCGATCTTAACCCAGCCCTCTTCACTTACCCTGAACTCAA
CGCCTCGAGGCTGGACCTGGAGCCCGAGAGAAGGACTGAACTTGGGTGGCCTGAAGAGCTAGCACACGCTGGTCAGCAGCT
GGGCAACGTCACTCTGTCCCCACCCTGACTCAAGTCTAAAAGACTGGCTTTTCCGAGAATGGGGTGTCGAGAGGGTGTGGG
GGGATGCGAGTGGCTGCCCTGCGCACTCTGCCAAGGCAGCATAAGAGCTGTTCTTCTGGTTTCCTTGGAGAAAAGCTCTGC
TGCCCTGATTATGAACTCTATAATAGAGTATATAGCTTTTGTACCTTTTTTACAGGAAGGTGACTTTCTGTAATCATGTGA
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c. 5′ probe

cctggcctgaaagatttcaagctgccagtacttataagggacaccgatgaatgcaaggccacactgggctacagag
ttagagtccaactcaaaaacacctacagcttcccctcaacaaaacaaatatctcacatggggttgatgctcagaaaatacc
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ggggtgtatatgtgtgcaaggctcttgagttctgtcccaagcacctacataaatacaccttatctgtcaccctacc

d. 3′ probe

ccaactcgggtttcctgactcggcccggaggcggcctgcatcgcaccctgctccttccttccttcattctttcagg
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