Osr2

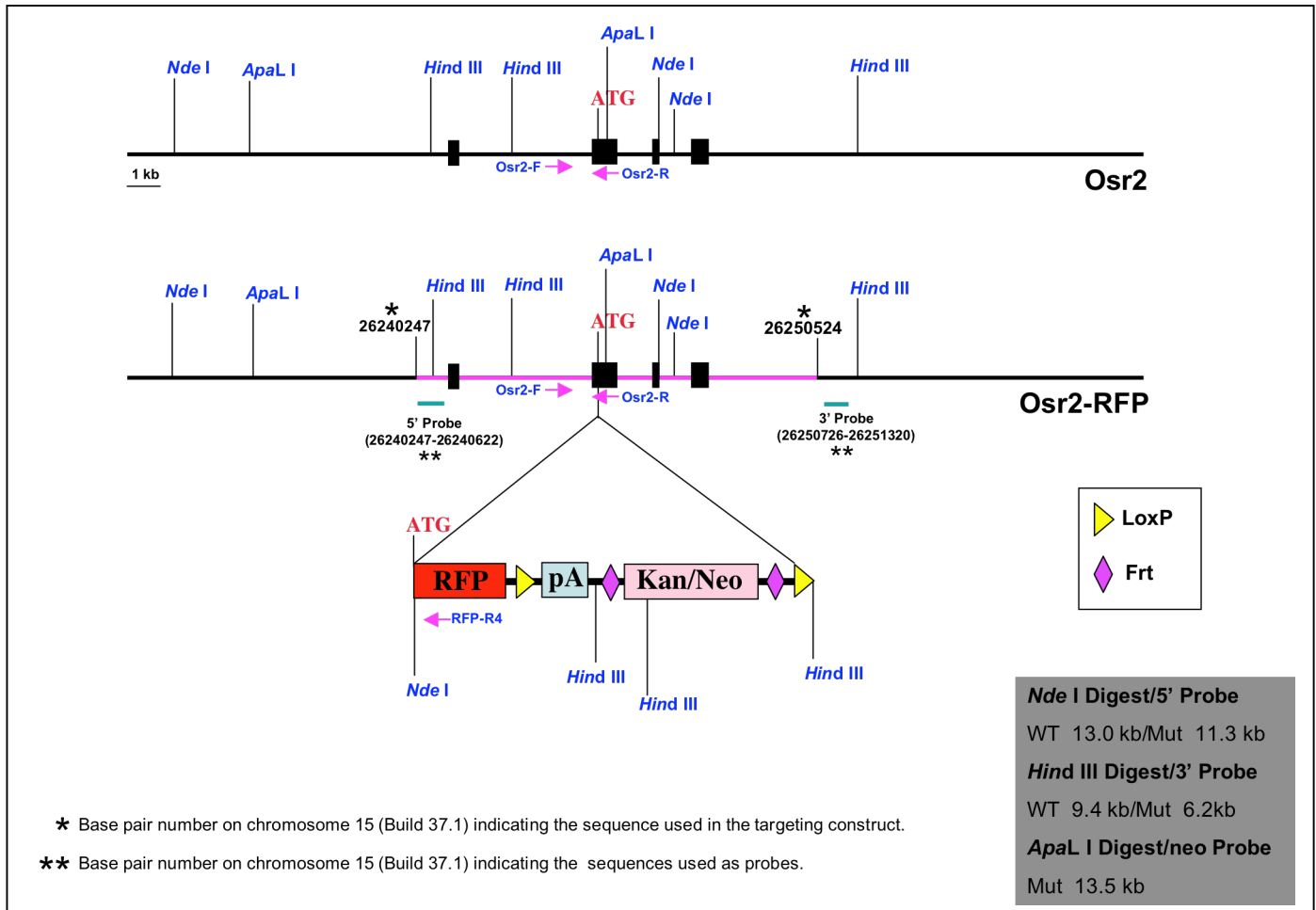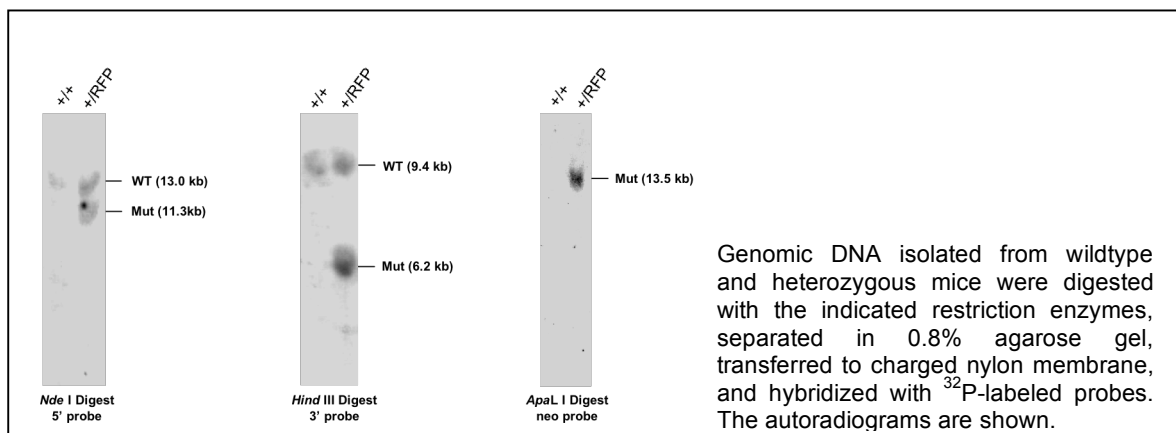## A. Rationale

Osr2 is expressed in the proximal region of the S-shaped body within the developing metanephros. An Osr2 knock-in approach was employed by the GUDMAP consortium to investigate the role of this population of cells in more detail.

B. Targeting Strategy

* Base pair number on chromosome 15 (Build 37.1) indicating the sequence used in the targeting construct.

** Base pair number on chromosome 15 (Build 37.1) indicating the sequences used as probes.

Nde I Digest/5' Probe
WT 13.0 kb/Mut 11.3 kb
Hind III Digest/3' Probe
WT 9.4 kb/Mut 6.2kb
ApaL I Digest/neo Probe
Mut 13.5 kb

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
Osr2-F: 5' cgaggtcttggaggctaagg 3'
Osr2-R: 5' ccctgaaggtggtccactgc 3'
RFP-R4: 5' agttcatcacgcgctcccac 3'

b. Expected Band Sizes
Osr2-F + Osr2-R: 347 bp
Osr2-F + RFP-R4: 529 bp

E. Relevant Sequences

a. Genomic clone used for targeting construct
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gtaggtagcctaggcctcgcaatatgcaaggacctcctgcagctcctttccttggcctcctcctgaatgctcacttctttc
cgctgctcttaaggcttcctggggggtgccagatcaggaagcagccccctttttctgagatcctagcgctcggtttgggaa
aacagggttccagactcaggttccgcatcctcacccggaggaaccatgctggagtctgaaccacctggaagggatttttggt
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cctgtgggcccgtcacttctgataaatgagagtaaggcggggggggggggggacaaaacaaaacaaaacaaacaaacaaaa
aaacagcgaggtcagcgtgaggagaagctgagccctgcaagcccgggagaacatagtgctcccggttagcgcgggaaaggc
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atgatttcccccacttaagaagatgcgggtgacagtgggactgactggtgcagcaccagagccctctctgcccgcctttctt
tccccggtcggagcccctgtcctggaacccggccttcctatcacccgctcgcccgagggcctcttgctgcccaagctctg
ggaatctcggtgcagactggcgggtctgatttcgaggtcttggaggctaaggaaaagggaatttaagagatgtcacaggcg
```
                              ———————————————————————————————▶
                                          Osr2-F

```
agatcgcccaaggactgaagaggctggataattaatcgtgatgtctccctctcttccccggacactttttagGGATTCCTCT
```
                                                                    ——————————————
                                                            Intron/exon junction

```
GCGTGCTGTTGTTTTGCGGGCTTTTCCCCCTCACACCCTCCGGCCTCTGAGACCTGGAAGAAAGGGGCGGCTCTCTGCACC
CCTTCGGAGCCTTCAGGAAG ATGcatatgGCCTCCTCCGAGGACGTCATCAAGGAGTTCATGCGCTTCAAGGTGCGCATG
```
                     ———————————————————▶
                       Start of RFP

                       —————————
                         Nsi I

```
GAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCAGACCGCCAAG
CTGAAGGTGACCAAGGGCGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCCAGTACGGCTCCAAGGCCTAC
GTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTC
```
                                                                  ◀——————————————
                                                                        RFP-R4

```
GAGGACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGC
ACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCACCGAGCGGATGTACCCCGAG
GACGGCGCCCTGAAGGGCGAGATCAAGATGAGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCCGAGGTCAAGACCACC
TACATGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAAGACCGACATCAAGCTGGACATCACCTCCCACAACGAGGAC
TACACCATCGTGGAACAGTACGAGCGCGCCGAGGGCCGCCACTCCACCGGCGCCTAA gaattcctgcagcccaattccga
```
                                                       ——————————————————————▶

End of RFP

tcatattcaataacccttaat ataacttcgtataatgtatgctatacgaagttat CTGCAGGCGCGCCCCTAGGGCGGC

——————————————————————————————————→
LoxP   Start of bGH polyA

CGCCACCTCGAGGGGGCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCC
TTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTG
TCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGC
GGTGGGCTCGAGATCCACTAGTTCTAGCCTCGAGGCTAGAGCGGCCGCCA ccgcggcgtagaggatctgttgatcagcag

——————————————————————→
End of bGH polyA

ttcaacctgttgatagtacgtactaagctctcatgtttcacgtactaagctctcatgtttaacgtactaagctctcatgtt
taacgaactaaaccctcatggctaacgtactaagctctcatggctaacgtactaagctctcatgtttcacgtactaagctc
tcatgtttgaacaataaaattaatatataaatcagcaacttaaatagcctctaaggttttaagtttttataagaaaaaaagaa
tatataaggcttttaaagcttttaaggtttaacggttgtggacaacaagccagggatgtaacgcactgagaagcccttaga
gcctctcaaagcaattttcagtgacacaggaacacttaacggctgacagaattagcttcacgctgccgcaagcactcaggg
cgcaagggctgctaaaggaagcggaacacgggatccatgcatagatcccccctcgaaaacgctagcgttaattaa
gaagttcctatactttttagagaataggaacttc AGCTTCTGATGGAATTAGAACTTGGCAAAACAATACTGAGAATGAA

←——————————————————————————————————
Frt   End of Kan^R

GTGTATGTGGAACAGAGGCTGCTGATCTCGTTCTTCAGGCTATGAAACTGACACATTTGGAAACCACAGTACTTAGAACAC
AAAGTGGGAATCAAGAGAAAAACAATGATCCCACGAGAGATCCATGCATAGATCTTAATTAATTAGAAAAACTCCATCGAG
CATCAAATGAAACTGCAATTTATTCATATCAGGATTATCAATACCATATTTTTGAAAAAGCCGTTTCTGTAATGAAGGAGA
AAACTCACCGAGGCAGTTCCATAGGATGGCAAGATCCTGGTATCGGTCTGCGATTCCGACTCGTCCAACATCAATACAACC
TATTAATTTCCCCTCGTCAAAAATAAGGTTATCAAGTGAGAAATCACCATGAGTGACGACTGAATCCGGTGAGAATGGCAA
AAGCTTATGCATTTCTTTCCAGACTTGTTCAACAGGCCAGCCATTACGCTCGTCATCAAAATCACTCGCATCAACCAAACC
GTTATTCATTCGTGATTGCGCCTGAGCGAGACGAAATACGCGATCGCTGTTAAAAGGACAATTACAAACAGGAATCGAATG
CAACCGGCGCAGGAACACTGCCAGCGCATCAACAATATTTTCACCTGAATCAGGATATTCTTCTAATACCTGGAATGCTGT
TTTCCCGGGGATCGCAGTGGTGAGTAACCATGCATCATCAGGAGTACGGATAAAATGCTTGATGGTCGGAAGAGGCATAAA
TTCCGTCAGCCAGTTTAGTCTGACCATCTCATCTGTAACATCATTGGCAACGCTACCTTTGCCATGTTTCAGAAACAACTC
TGGCGCATCGGGCTTCCCATACAATCGATAGATTGTCGCACCTGATTGCCCGACATTATCGCGAGCCCATTTATACCCATA
TAAATCAGCATCCATGTTGGAATTTAATCGCGGCCTCGAGCAAGACGTTTCCCGTTGAATATGGCTCATATGAAACGATCC
TGTCTCTTGATCAGATCTTGATCCCCTGCGCCATCAGATCCTTGGCGGCAAGAAAGCCATCCAGTTTACTTTGCAGGGCTT
CCCAACCTTACCAGAGGGCGCCCCAGCTGGCAATTCCGGTTCGCTTGCTGTTAATTAAGCGGCCGCTCTAGCCTCGAGGCT

AGAACTAGTGGATCTCGAGCCCACCGCATCCCCAGCATGCCTGCT ATTGTCTTCCCAATCCTCCCCCTTGCTGTCCTGC

←——————————————————   ←————————————————
Start of Kan^R   End of bGH polyA

CCCACCCCCACCCCCCAGAATAGAATGACACCTACTCAGACAATGCGATGCAATTTCCTCATTTTATTAGGAAAGGACAGTG
GGAGTGGCACCTTCCAGGGTCAAGGAAGGCACGGGGGAGGGGCAAACAACAGATGGCTGGCAACTAGAAGGCACAGTCGAG
GCTGATCAGCGAGCTCTAGAGAATTGATCCCCCC TCAGAAGAACTCGTCAAGAAGGCGATAGAAGGCGATGCGCTGCGAA

←————————————————   ←————————————
Start of bGH polyA   End of Neo

TCGGGAGCGGCGATACCGTAAAGCACGAGGAAGCGGTCAGCCCATTCGCCGCCAAGCTCTTCAGCAATATCACGGGTAGCC
AACGCTATGTCCTGATAGCGGTCCGCCACACCCAGCCGGCCACAGTCGATGAATCCAGAAAAGCGGCCATTTTCCACCATG
ATATTCGGCAAGCAGGCATCGCCATGGGTCACGACGAGATCCTCGCCGTCGGGCATGCGCGCCTTGAGCCTGGCGAACAGT
TCGGCTGGCGCGAGCCCCTGATGCTCTTCGTCCAGATCATCCTGATCGACAAGACCGGCTTCCATCCGAGTACGTGCTCGC
TCGATGCGATGTTTCGCTTGGTGGTCGAATGGGCAGGTAGCCGGATCAAGCGTATGCAGCCGCCGCATTGCATCAGCCATG
ATGGATACTTTCTCGGCAGGAGCAAGGTGAGATGACAGGAGATCCTGCCCCGGCACTTCGCCCAATAGCAGCCAGTCCCTT
CCCGCTTCAGTGACAACGTCGAGCACAGCTGCGCAAGGAACGCCCGTCGTGGCCAGCCACGATAGCCGCGCTGCCTCGTCC
TGCAGTTCATTCAGGGCACCGGACAGGTCGGTCTTGACAAAAAGAACCGGGCGCCCCTGCGCTGACAGCCGGAACACGGCG
GCATCAGAGCAGCCGATTGTCTGTTGTGCCCAGTCATAGCCGAATAGCCTCTCCACCCAAGCGGCCGGAGAACCTGCGTGC
AATCCATCTTGTTCAATGGCCGATCCCATATTGGCTGCAGGTCGAAAGGCCCGGAGATGAGGAAGAGGAGAACAGCGCGGC
AGACGTGCGCTTTTGAAGCGTGCGAGAATGCCGGGCCTCCGGAGGACCTTCGGGCGCCCGCCCCGCCCCTGAGCCCGCCCC
TGAGCCCGCCCCCGGACCCACCCCTTCCCAGCCTCTGAGCCCAGAAAGCGAAGGAGCAAAGCTGCTATTGGCCGCTGCCCC
AAAGGCCTACCCGCTTCCAT tgctcagcggtgctgtccatctgcacgagactagtgagacgtgctacttccatttgtcac

←——————————————   ←————————————
Start of Neo   End of PGK promoter

Gtcctgcacgacgcgagctgcggggcggggggggaacttcctgactaggggaggagtagaaggtggcgcgaaggggccacca
aagaacggagccggttggcgcctaccggtggatgtggaatgtgtgcgaggccagaggccacttgtgtagcgccaagtgcca
gcggggctgctaaagcgcatgctccagactgccttgggaaaagcgcctcccctacccggtag aattcga gaagttccta

                                ←

                   Start of PGK promoter

tactttttagagaataggaacttc gatcc ataacttcgtataatgtatgctatacgaagttat tagtccctcgagggg

        Frt   LoxP

atccactagttctagag GGGAGCAAGGCCCTGCCAGCTCCCATCCCACTGCACCCGTCACTGCAGCTCACCAATTACTCC

              →

         Osr2 continues

TTCCTGCAGGCAGTGAACACCTTCCCGGCCGCAGTGGACCACCTTCAGGGCCTCTACGGTCTCAGTGCGGTGCAGA CCATG

               ←

                Osr2-R

CACATGAACCACTGGACGCTGGGGTACCCCAGTGTGCACGAGATCACCCGCTCCACCATCACGGAGATGGCGGCTGCGCAG
GGCCTCGTGGACGCTCGCTTCCCCTTCCCCTCGCTGCCCTTTGCCACCCACCTGTTCCACCCCAAGCAGGGGGCCATTGCC
CACGTCCTTCCAGCCCTACACAAGGACCGGCCCCGTTTTGACTTTGCCAACTTGGCGGTGGCCGCCACGCAAGAAGATCCT
CCTAAAATGGGAGACCTGAGCAAGCTGAGCCCAGGGCTGGGGAGCCCCATCTCGGGCCTCAGTAAACTGAACCCGGACAGA
AAGCCCTCCCGAGGGAGATTGCCCTCCAAAACGAAAAAGGAGTTTATCTGCAAGTTTTGCGGCAGACACTTTACCAAATCA
TACAATTTGCTCATTCACGAGAGGACCCACACAGACGAGAGGCCATACACCTGTGACATCTGCCACAAGGCATTTCGGAGG
CAAGATCACCTACGGGATCACAGgtgaggttggggactatggcaaggtgaatctgcccatgaaatgtcctattagatattc

            Exon/intron junction

caagtgtcactaaaattcccctctattatagaatgctcatccaaacccctcccaaagctgacctcagtaacccaccccca
gtctatttaccgccctccgaacctcgctgaggaccgctgcattctttgaaacaagaaagagctttcctctgtccttgagca
ggaaaaatctggcctaggagtcagtaactgtttcctctgagtcggccttggtactgagtgagaggtgaaaaggctgcggga
tcgcggattccttccagctggaggggtggctgaaggatgcaagtttgggctgctgtggcgccatttccggacaatctaccg
gattcttctacaccgccacccccctccgccccaacacacctccccgagtgccctggagctcaggtttttcttattctttgtg
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ttgccatggctggaggtttcgcctaaatctacctgtaacttctgaggtgggacttcatccttgaggccaagatatgattcg
caaaaatcgtctcccaaccccagccttgccttctgtcttgatttagtcattttaaatcccagagaaaaccctgacggcct
agagggctctctctgctgcattgccagctccacaaacttgccagagaggttggtcaagggaacataaagccagactgctcg
tgaatgtttccaagagtctgttagttgaggttaaccataaacagaattatacagaaaatctaaaacaacataagtaataga
aattctgctttctttgccctctcaaaatttcgttggagaaattgtggaagaatgatcctttcaatgaaaacttgtattga
ttttcagGTATATCCATTCCAAAGAAAAGCCCTTCAAATGTCAGGAGTGTGGGAAAGGATTCTGTCAGTCTAGGACCCTAG
CAGTTCACAAAACTTTACATATGCAGgtaagtttatttttcttcttttaaaagtgcatgtttttattttaccattatggctt
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gtttttaaaatataaaattaacattatgtaacataaagaatgtttcttaacgaacagcccagccgattaacaataagtcct
aatgttagtattatatatatattttttttcatcccatctgttctgcgtttaaaacttccgtgtttgggaggcaataaaggta
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accccccctttcctgcccttgacgccgcgatgaaatacgtggcccaccgaggtggtcttatcagtgcaatcgggcctctgc
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gcttgttattttaaggcttgatacgggtaagacagaaactgttgaggagggtgccgtggtggtgtgaaggattaatccttt
gccttgcttcacatctgaacaggaatctccacacaaatgtcccacatgtggaagaacctttaatcagagaagtaacctgaa
aactcaccttctcacccatacagACATCAAGCCCTACAGCTGCGAGCAGTGCGGCAAAGTGTTCAGGCGAAACTGCGATCT
GCGGCGGCACAGCCtgaCTCACACCCCGCGGCAGAACTTCTAGCGAAGCCCAGGATCTGTCCTGTGCGGCCCCGGCTCCCT
TCCCCCCAGACACCTCTCCTCGCCTCCCACCCCAGGGGCCCATCCCTACCCTCACTGACCCCGGCTCTTCCCTGCCTGCAG
CCGCACCTGCAGCTCCAGGGAGTTAACTGTTCTTCTGAGGACTGAGAACTCTCTAGAAAGCCACACACTACCCCCTTCCAA
ACCAGCATGTGCTAGATCCTTGTAGATATTCACGGCTCATTTTAGAGCTCTGTACATAATATTGTGGGTCTTCGTCCGGTC
GTTCTGTCTGTTTTTGTATCTTGTTGGATGCACCTAGACATGGAAAACGAAACGCAAATTTATCTTTAAAGACTGTATTTT
CAAAATAAAACCTTTCTTGATTCAgtgcttcttgagattcatcttgctagccaatccggtggggttctggaggagtggacaa
cccctgcagactcaatggagaggatgttctttattcctaagtttttaaaaacaggacttccagttctcttaataaaatttga
cacccccccccttttttgtacaggggcgtttggaataggatcgtgccctttttctttttttcaaatatataaaattgaaca
gccttagggtctatccctcttgttaattctagtctcttgtgtttgttaagtttactccaggtaagtatactatagtaacca
ttgttgataacacactgttacacccttgaaaggcctcatggcaaaaaacacaactagaggagagaagggagcttggtgttc
tggaagaagtccttgacctgttgttgaagtttatggtgggcaacttttcctactattagactttgaacactgtgattctca
ctataaacacagactttatcctgccttcagggaacgagtataaaattctcccgcagaacaggaagatagcgaggatggggg
aagaggaggaaggagaaactacttagttgtgtttgcacacaatatttctctctaagggtggagatcaattctgtcagaagt

ttaggatttctccacgaagcaaagggagtgaggtcactgcctgagccttacccttgagaaaaggtcaagtgtagtctgctc
cactccacgaatggagcctgcagtagacacatggtagccctgggagcttgtcagggtttcccaagtaggtctggagctcag
aatactaggacactgacaatcctgaagtcagtagaatttggtgggaggctctgccgcagaggtaaacaacagtatgttgcc
aaacggcagggaaatgacccaatttagagaacatctgcagaacctgtggcccagtggcccagtaggtgctcatacctagtt
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accttcacgggatagcaagcaatatgtcctttctttatttgataccttaagatcctccagaataatagatggggatttgaa
gttaagatttgaaatttctcactagtgtctgtttgatatcatttcctgggaggtttcaacacatttcagattcacccccctt
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tcaagattctgcctgccttgacttggaagatatgagtactttagggcagggttaacatggactgtccctagaggaaacccc
cctaatctttgctgcttttccatgcagtcagactcacagccccacccacccttacatactacaagtgtcatagctccact
gtacagacatcagctgaagtaggaaagaatgacccttgctagaaccaagttgcaagcatgttctggaggtcccaccacaga
tatgtagattgatataggttactgttggtttccaattattctagttaattaaataagactcataagtgaatctggaggccc
agaagagaaagccaagaaggcagttcctcttcccatccacacaccatttcaaaagtcaaggtcagttcacatacaatgtat
ttccttattccttaatttaacaagtgtttgttgcgtgcttttcagttgccaaACCTTATGGCGGTTGCCACT

c. 5' probe

ccagctaaagtgtcctcaagtggtttgggacgagggacagtttgcttagttctgttccggcggcgcgttgcgtctg
aaaagaagttgcttcaagtggatcttatcgttccctgacatccttttccagagggactgtaataccctctcatttacacgc
tcacaaaactaaacctttaggagaactaagaaacctaacagtacatgtcaaattcaatcaattttcctggcggctgaacaa
tgaactcaattttgctgagaatgaagataatgcacgttttgaggcactggaatagttttttcctaaaatttaaaaaagat
tgttcttcacccattccagatgggcaggttattgtttgccaatggattgcagatgcc

d. 3' probe

ggcacatttaggaatcctgactacctaactattttaaaatctatgtgatagagcttaatttaatagctattaaat
ccactgaccatctaggctcaggcaggactatactttgtcatttgacttgaaacattcttcatcattccaggtgaaattctg
aatacttacttcctctccaatctctgaggttttgttttttttttgtttttttttttcctcctctatgcacaagtgtgcagg
aaatgcatctgtgtgagacactctcataagacactgtaaggacaaggcacatcttgtgtcttaggaaatagattttttttt
tttgagttcagggaagactcattatttatgttttagattagtccgtgtttccttacaccctcgatcatagttaacttatga
atcaattgccttggctaaaggttcagaggaggaaagatcaccagtcatagccacagtgtagaaaaaaatcaacgaaccgca
gttacaaatgatgattgagctgcaactgtagaatcttctcatctgtgagagatgaaaaaccatttctgaacactaattaca
ttgtaaccagttgcctgggaggacttctgagtg