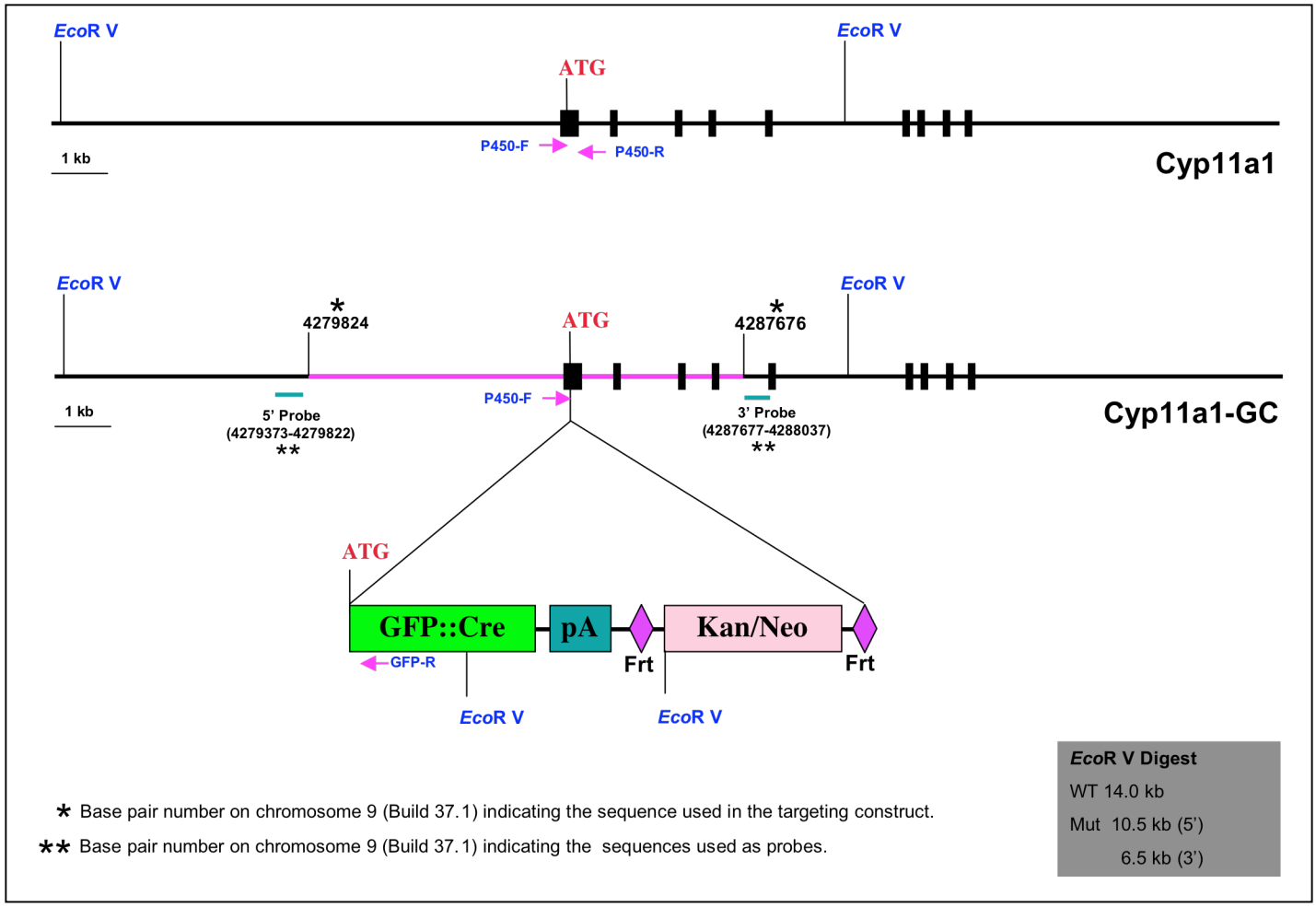
Cyp11a1

A. Rationale

For Leydig cells, a specific (with respect to testes) and early marker is Cyp11a1 (P450scc). There is a strong consensus for this strain.

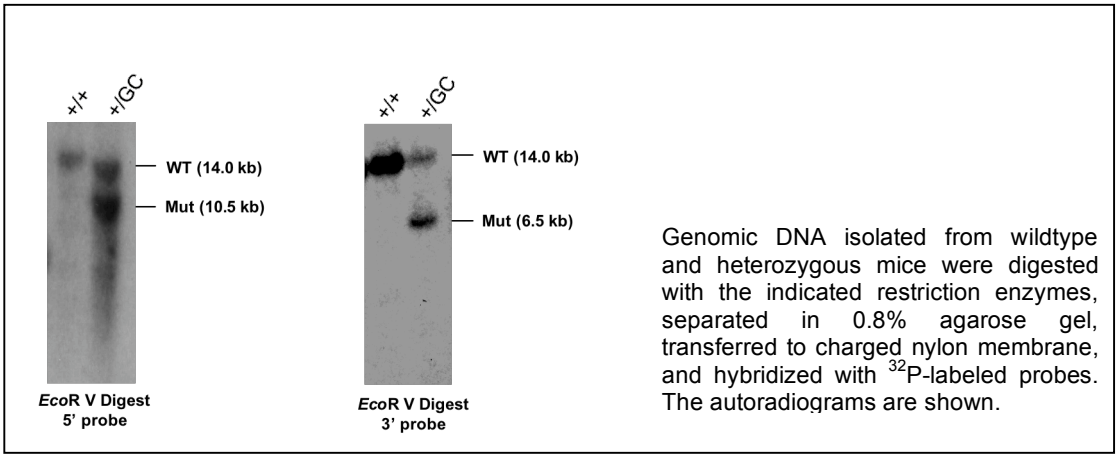
B. Targeting Strategy



* Base pair number on chromosome 9 (Build 37.1) indicating the sequence used in the targeting construct.

** Base pair number on chromosome 9 (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

P450-F: 5' gagctgcctgccagtgtttg 3'
P450-R: 5' ggacctaggactgctagtag 3'
GFP-R: 5’ gtccagctcgaccaggatgg 3’

b. Expected Band Sizes

P450-F + P450-R: 376 bp
P450-F + GFP-R: 296 bp

E. Relevant Sequences

a. Genomic clone used for targeting construct

ccctaatgaatacacttccatcgggtgggcttagttctgttcatttaacaactctcagtgactgggggatgcattc
tgggaaggcggaggggcagggtgtgtggaggagctggcctagcctgggaggatgggtctgggttaggtttgatgggggag
aaactgagctgcagagcttgtggtagctccgtttgccaaggtctgggagccgacatacagcctgaccatggatcttagctt
agtggacactgaatccttcggagtcagtcattctgaccccgatttattgggtgagagataaagaggaatctggttctaggg
tgaaattcctttgcttctccaaatctccccaaggtcagaggagattaaagtttccccgtccgcccctggccttgtcctccg
acatcaactggaattctcagcagaagtcggctgtgactggaggctagcctcacacagggacctgggggggaaatctcttgat
tggaccttgtggctctgtgggaagccagagacctgatccctgcttactctagtgacccatgatgggtatctgcctcttcct
acctctgcctcatttttctcatctgtacaatgggagctgagagaaatgagcctgtgacatccggtaattcagccagcagct
tctggtgtgcagggtgtacccttataatgctaactgcccttactcatatcccaggtctgcttctcagcagcatggtgaaag
gggcttgagagccgagtccccagccaggaacaacttggacttgaatccatactgtgttgctagttagcattgtgactctgg
gcaagttgctcttttggttgagctttagtctcctcctctgtaactctggcaaagtttctgaacctacctggcaagggagcc
atgaggatgaagaactgtgcaaagctccttagacatggcaagcgtccagttcattccacagacacaggtgaggcctacggg
tcctggccaaggctgagaaagccaagtgctctttctaacatttgacatcatcatccctagggcattgatcccagagaggtt
aagacagagctcctggttgcaggcatcttgatttctgttcgatgactagtctgctactcacagaaagcagcttgaaagggc
tttgaaaggaatccagggccaaggatctggaggccagggtaccagaagccaatgggaccaggcccatggcctacttactgc
ggcttatcctttctttatcagattcccagacccacagaggttaagacagccccagatgcaaattcatcccccagtgagtca
ctgtctttgtgcagccaaggtttctcctcccccatcctgagcccaacttaacattgctgtggaatcatggaagaactttc
tatgaagaaggtggtctcctcttgcctccatgggctctgactctctgttccctcacccctctccagcctgactcacctgct
ccccctctccctcccacctggcttcttcgtttccttctgctgttttctgccagcctgggcttcccttagttttctcagaaga
agggcagggagtttctaccctgagaagaggcatgggaattcacacctagacagcccagcccaggatacccagctgttttga
tgaaagccaaggctgctaattggcccaggaagttgagtcagatggctgccaacctaaattagcaggaatgctgttcaggcg
tgatgggttgttatccttgctagaacccagtgtaatgaacttctgtcccttcgctgacaatatgctagtgacaacacaggg
ccataggagtccaatttcctcccacattcagattcacattccctctcctctaccctccctcctctcccatcccttccacc
ttttgagacagactctgatatagcccaggctgacttcaagttcatggccgaggaccgctgtgaacttccatacctcatgcc
ttctgcactgggggaagggcacggtcagggtggactgccctcctcccacagtgacaatccactttccttctcacaatccta
aggagggcaaaggctgcactaaaacttagcttaggtttaattcaatgaactggggttttcttttaaagacaccctgcttt
tcataaggaaaataatgtgttagaaagaccacaaatttttcttagcctttacgtggaataacattcaataaaatggaaaac
aagcaaaccgacaagagctaggcatggttgtgcaaccgtcaatcccagcattcaggtggctcactgtgagttcaaggccaa
cctgatctacatagagagttccaggttagctggggctacaccgtgattccctattaaatccatgctaagggtgtatgtagc
tcagtgagttttgacatatgtaagaggtagcagaaaaagaacatcctgagctggatgtggtagaacataggtacctgtaat
ctcagcgtttgagaggaagagaaaggaaggtcacctaggctagaccatatggtaagatcctgttttaaaggcaaaggaagg
ttacctaggctagaccatatggtaagatcctgttttaaaggcaaaagaagaaagtacactttctgtcatagtggtgaatgc
atataatcccaggactcaggacactgaggcaggggatcttaaaagcataatgaaaataaaaaatgcactaaatcctggag
agatggttcagtggttaagagcactgctagtgctttgactgttcttccagaggatccaggttcaaatcccagcacccacac
ggcagctcaactatctgtaagtccaagatttgacactttcaaacagacatacatgtaggtcaaacaccaacgcacattaaa
tagataaaaaaacaacataaataaatacatacatacataagtaaataaaacaatacttgctcgtcagaacattttcaaaga
acacggagtatggttgtttgtcaaagagaaaaaaaaaagcagaatttcaagaattaaggaactgtacaagatcagttaata
atcaaccatttgtaagtcccaggaacacaggaaagatgtctgctgttctataactggccaggtagtcagttcctccagacc
cgtgcatgatcttaggctatctctcatcttagaggatattcgtcatcacctaggggccagcagaaggcctttgtcatgcaa
aacccgcaaggaatggaagcaaacccaactattgcagagtcataaaggggcaggagctgaggttgagacccaagggggggg
gggctagccatgagccaggaggtctaagcctcattttcttcccatgaaaatggagatagcaaagttgccttttaatgtgtc
tgaaggatagtggtcttagggacccatagggtggacactgaggttagaggcctg<u>gagctgcctgccagtgtttg</u>cctaact

P450-F

ctactacagacttttcagacagaaagttcagaagttctttctctgagtttgggagGGGCTGTGTGAGCCCAGCCCTCTGGA
GGAGGAAGGGGGGAGGTCACCGCTCCATCAGCTTCTCTCTTAGCCTTGAGCTGGTGGTTATAAGTGTGGCTCTGGAGGTTT
GGGGCAGAGACACTCGTGCAGCAGGAAGTGGCAGTCGTGGGGACAGTATGCTGGCTAAAGGACTTTCCCTGCGCTCAGTGC
TGGTCAAAGGCTGCCAACCTTTCCTGAGCCCTACGTGGCAGGGTCCAGTGCTGAGTACTGGAAAGGGAGCTGGTACCTCTA

CTAGCAGTCCTAGGTCCTTCAATGAGATCCCTTCCCCTGGCGACAATGGTTGGCTAAACCTGTACCACTTCTGGAGGGAGA

P450-R

GTGGCACACAGAAAATCCATTACCATCAGATGCAGAGTTTCCAAAAGTATGGCCCCATTTACAGgtaagcctggcagcagg
tggagcaggcacaggggaggcacacggctcctgcttcctgtctgccttttttctttcatttcttccaggctggggctgggg
ctggggctggggctggggctggggctggggctggggctggggctggggctggggctggggctggggctggggctggggctg
gggctggggctggggctgaggggatagtcttccctcatttctgactccaagacttttctactggtataaagaagtgtgag
caagcagtgccccccctcacccaccaactttagccaccctaaaagtcaagtttgctgtctctatcttcaggggaaggaaat
gagacattggtgccctgactactggcaagccccgaccaggctttagcgccagcctcagcttcaggccctttgtgtctggga
gctgagcaagaacagtacacgctcttaatcactgagccatctccccaccttgacatttttctttaatagaagtgaagggtg
ccatctcacttttgaggcgagtgactactctgaattacaattcaagaagagccgggctggcttggaacttgggagatacag
ggcattcactgtagaatgacaagaaagcctgccatggcttcaagcccagcaggacaagaaggttcagcgtggaatcggcat
gattatgaaatggaaggattggggtgggagtggggctggtcctggggtgcgctgtgtggtgtatgatagtgtcctgccgtc
ctttggaatgagtcacaatgtcataggaaaatgtgtcatgtttggctcttcagcagctccgcaagacagatgtcacatcca
tttcccatctcataggtgacactgaggctcagcaaagggaaggtcccagccagaaagctgtgtactgattgtgtagagagg
cctggctgcccagggtgtgtgttcacggctgcctcaggtccaacatcagccgctcttgtctttccagggagaagctgggca
ctttggagtcagtttacatcgtggaccccaaggatgcgtcgatactcttctcatgcgagggtcccaacccggagcggttcc
ttgtgccccccctgggtggcctatcaccagtattatcagaggcccattggggtcctgtttaagtgagttctgagtgagcctc
gtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtagggtgctgaaggaaagtggtagggagaggggtcg
ggggaaagagtggctctgctcatcttcctgttccataaggcaaacgatggtgacaacagccatgacagggaagtgtcctgc
cgactcccaggggatgtcaggctgaagttcttcatgaatagtggagactttgcaaagttctcactaattgctttaactat
gaagtgccactttatcaaacacctgctaggtaccaggccttgtgccaggtgttggcagtacagctgtaaataagggcctga
cagcctgaagccactgtgagaaacagcgagatctgccactgagatatagatgctattttctaccaaattgcatactgataa
atttatatatatttatatataatttttatataaatatataatttatatataaatataaataacatataaaatatctatcta
tctatgtatctgtctatgtttctacattgatagggtctttctacattatctagaactttctagaagttgctacatagacca
ggctggcctcaaacaaacatcagcttgctttggcctcctttaaaggttaaggacatgtgccaccacacccagtgtcactga
aacttttaaatgtgactaaatcaagggttggcaaggccctggagcaactggagctctcgtgcactgtggtgggaacatgta
cctggcctctgtgtgaacagtgcttggatgttaactcccaagaccgactattaccagggccacggtggctgtgagtcacac
agtgtttctaactttagtgacatgccatgtcctctcagggaacagggtcataggctagaatgtttctccaggctatagtta
aaaaaaaaagaaagaaaaaagaaggaaagaaaaagaaaaaccaggaagccttctagctggttgagatgaaggaggacggtg
gacgagagaaggttgcatatacctgccacaatgtacaggtacagactcaggtccacgacacggctgtcctgaaatgcaaaa
ctgagtggagaaggcaagtactccaaactctgacaggcatttagaaactctggagctagcacagtctttttagtgtctttt
aagagagattcagtaaaaccatacacaaaaggggaagggacccaggctcgggtgggggcttcatgtggagcagggtagg
ctgaaaccatgagtttacaaaattatttgcctccagactctcaacttttaaaagaagtttttattcatagaattctattt
gtgtgtatatgtctggtaagtatatgccatgtgtatacagatgcctctggggactaggagagggcaccggatcccctgtgg
ttgttacaagtggtccagagttgccctatagggatgctgggaactggacttgattcctctctaagagcagtgagccatctt
gccagctcacaacgttcttaaacaaacaaactcccccaaattacaaaaggagcattgcagccgggcagtggtggtgcacac
ctctaatcccagcacttgggaggcaggggcaggcagatctctgagtctgagaccaggcagggcagggagggagtaggagag
gagagcaaatgttgggaggaggggagatgatgttgctgcaggtgctggagctagagagcctagcaagtccgtaggtgccag
gtgtggcatctgtgaccttgaggcgggacctgtgtttctctaggagttcagatgcctggaagaaagaccgaatcgtcctaa
accaagaggtgatggcgcctggagccatcaagaacttcgtgcccctgctggaaggtgtagctcaggacttcatcaaagtct
tacacagacgcatcaagcagcaaaattctggaaatttctcaggggtcatcagtgatgacctattccgcttttcctttgagt
gtaaggatcttacacatgactgactggcaggagctgggggtggggagccagcgttcacccttttaccttgagaccccaggc
cctgtgcttgtgtggattggtttcttggacagactctgtactgtcagtctctcgggggtagtcaaatagctctcatctgac
attcatgttggggaagtccccagatcataggttttccatgccaccatcctccataagccagaacttggcttggccctgcat
gctgcaagtctccctctcgtagaaacagacctctggagttgagcaagaaagaggggacctcctgggttgatctgggctgac
tgttgaaggacacttagaaattatccagactgggtaaggcggttaggcgtcatagaccaggcatagaacagtgcatgcata
tggcttagtagaagtcatgtgatgcctgcaggagatctggggtaccctgggcttggtataaaattggcattcaaatttttc
ttgtggtaagtgagaaaacttgaatcttttagtttcagccagattgaatcctgggtgacttggagactgctaacctaagtc
tcccaggccatgtgtgatgtagtgattgcagtcagtaacaaagggaaatctctcctggacagttccgtcctggctcaggtg
gagtccactattcccatcaaagcgtcctgatcagggccgcccctatatccctctgagggacctgaattgaagccagaaggg
cagtcttcttccatctcttggggctcaggtctcagttgctttgctggaggaagctaaggatgagcaagggcccctgtgtgt
ggtgggcatggatggggatggttctgggtgctgaggctcttggagagccaggctggaagagggtccttagcctcagtctct
c

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

ccctaatgaatacacttccatcgggtgggcttagttctgttcatttaacaactctcagtgactgggggatgcattc
tgggaaggcggaggggcagggtgtgtggaggagctggcctagcctgggaggatgggtctgggttaggtttgatgggggag
aaactgagctgcagagcttgtggtagctccgtttgccaaggtctgggagccgacatacagcctgaccatggatcttagctt
agtggacactgaatccttcggagtcagtcattctgaccccgatttattgggtgagagataaagaggaatctggttctaggg
tgaaattcctttgcttctccaaatctccccaaggtcagaggagattaaagtttccccgtccgcccctggccttgtcctccg
acatcaactggaattctcagcagaagtcggctgtgactggaggctagcctcacacagggacctgggggggaaatctcttgat
tggaccttgtggctctgtgggaagccagagacctgatccctgcttactctagtgacccatgatgggtatctgcctcttcct
acctctgcctcatttttctcatctgtacaatgggagctgagagaaatgagcctgtgacatccggtaattcagccagcagct
tctggtgtgcagggtgtacccttataatgctaactgcccttactcatatcccaggtctgcttctcagcagcatggtgaaag
gggcttgagagccgagtccccagccaggaacaacttggacttgaatccatactgtgttgctagttagcattgtgactctgg
gcaagttgctcttttggttgagctttagtctcctcctctgtaactctggcaaagtttctgaacctacctggcaagggagcc
atgaggatgaagaactgtgcaaagctccttagacatggcaagcgtccagttcattccacagacacaggtgaggcctacggg
tcctggccaaggctgagaaagccaagtgctctttctaacatttgacatcatcatccctagggcattgatcccagagaggtt
aagacagagctcctggttgcaggcatcttgatttctgttcgatgactagtctgctactcacagaaagcagcttgaaagggc
tttgaaaggaatccagggccaaggatctggaggccagggtaccagaagccaatgggaccaggcccatggcctacttactgc
ggcttatcctttctttatcagattcccagacccacagaggttaagacagccccagatgcaaattcatcccccagtgagtca
ctgtctttgtgcagccaaggtttctcctcccccccatcctgagcccaacttaacattgctgtggaatcatggaagaactttc
tatgaagaaggtggtctcctcttgcctccatgggctctgactctctgttccctcacccctctccagcctgactcacctgct
ccccctctccctcccacctggcttcttcgtttccttctgctgttttctgccagcctgggcttcccttagttttctcagaaga
agggcagggagtttctaccctgagaagaggcatgggaattcacacctagacagcccagcccaggatacccagctgttttga
tgaaagccaaggctgctaattggcccaggaagttgagtcagatggctgccaacctaaattagcaggaatgctgttcaggcg
tgatgggttgttatccttgctagaacccagtgtaatgaacttctgtcccttcgctgacaatatgctagtgacaacacaggg
ccataggagtccaatttcctcccacattcagattcacattccctctcctctaccctccctcctctcccatcccttccacc
ttttgagacagactctgatatagcccaggctgacttcaagttcatggccgaggaccgctgtgaacttccatacctcatgcc
ttctgcactgggggaagggcacggtcagggtggactgccctcctcccacagtgacaatccactttccttctcacaatccta
aggagggcaaaggctgcactaaaacttagcttaggtttaattcaatgaactggggttttcttttaaagacaccctgcttt
tcataaggaaaataatgtgttagaaagaccacaaatttttcttagcctttacgtggaataacattcaataaaatggaaaac
aagcaaaccgacaagagctaggcatggttgtgcaaccgtcaatcccagcattcaggtggctcactgtgagttcaaggccaa
cctgatctacatagagagttccaggttagctggggctacaccgtgattccctattaaatccatgctaagggtgtatgtagc
tcagtgagttttgacatatgtaagaggtagcagaaaaagaacatcctgagctggatgtggtagaacataggtacctgtaat
ctcagcgtttgagaggaagagaaaggaaggtcacctaggctagaccatatggtaagatcctgttttaaaggcaaaggaagg
ttacctaggctagaccatatggtaagatcctgttttaaaggcaaaagaagaaagtacactttctgtcatagtggtgaatgc
atataatcccaggactcaggacactgaggcaggggatcttaaaagcataatgaaaataaaaaatgcactaaatcctggag
agatggttcagtggttaagagcactgctagtgctttgactgttcttccagaggatccaggttcaaatcccagcacccacac
ggcagctcaactatctgtaagtccaagatttgacactttcaaacagacatacatgtaggtcaaacaccaacgcacattaaa
tagataaaaaaacaacataaataaatacatacatacataagtaaataaaacaatacttgctcgtcagaacattttcaaaga
acacggagtatggttgtttgtcaaagagaaaaaaaaaagcagaatttcaagaattaaggaactgtacaagatcagttaata
atcaaccatttgtaagtcccaggaacacaggaaagatgtctgctgttctataactggccaggtagtcagttcctccagacc
cgtgcatgatcttaggctatctctcatcttagaggatattcgtcatcacctaggggccagcagaaggcctttgtcatgcaa
aacccgcaaggaatggaagcaaacccaactattgcagagtcataaaggggcaggagctgaggttgagacccaaggggggg
gggctagccatgagccaggaggtctaagcctcattttcttcccatgaaaatggagatagcaaagttgccttttaatgtgtc
tgaaggatagtggtcttagggacccatagggtggacactgaggttagaggcctg**gagctgcctgccagtgtttg**cctaact

P450-F

ctactacagacttttcagacagaaagttcagaagttctttctctgagtttgggagGGGCTGTGTGAGCCCAGCCCTCTGGA

Start of P450 transcription

GGAGGAAGGGGGGAGGTCACCGCTCCATCAGCTTCTCTCTTAGCCTTGAGCTGGTGGTTATAAGTGTGGCTCTGGAGGTTT
GGGGCAGAGACACTCGTGCAGCAGGAAGTGGCAGTCGTGGGGACAGT ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGG

Start of GFP

GTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCC

GFP-R

ACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTG
ACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGC

TACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACC
CTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTAC
AACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAG
GACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCAC
TACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCC
GCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTCCGGTACAGCTCTCGACGGAGAAAGCTCAGGCTCTGGCTCAGAG
TCTGACTCC ATGGCCAATTTACTGACCGTACACCAAAATTTGCCTGCATTACCGGTCGATGCAACGAGTGATGAGTGTCG

End of GFP

Start of Cre

CAAGAACCTGATGGACATGTTCAGGGATCGCCAGGCGTTTTCTGAGCATACCTGGAAAATGCTTCTGTCCGTTTGCCGGTC
GTGGGCGGCATGGTGCAAGTTGAATAACCGGAAATGGTTTCCCGCAGAACCTGAAGATGTTCGCGATTATCTTCTATATCT
TCAGGCGCGCGGTCTGGCAGTAAAAACTATCCAGCAACATTTGGGCCAGCTAAACATGCTTCATCGTCGGTCCGGGCTGCC
ACGACCAAGTGACAGCAATGCTGTTTCACTGGTTATGCGGCGGATCCGAAAAGAAAACGTTGATGCCGGTGAACGTGCAAA
ACAGGCTCTAGCGTTCGAACGCACTGATTTCGACCAGGTTCGTTCACTCATGGAAAATAGCGATCGCTGCCAGGATATACG
TAATCTGGCATTTCTGGGGATTGCTTATAACACCCTGTTACGTATAGCCGAAATTGCCAGGATCAGGGTTAAAGATATCTC
ACGTACTGACGGTGGGAGAATGTTAATCCATATTGGCAGAACGAAAACGCTGGTTAGCACCGCAGGTGTAGAGAAGGCACT
TAGCCTGGGGGTAACTAAACTGGTCGAGCGATGGATTTCCGTCTCTGGTGTAGCTGATGATCCGAATAACTACCTGTTTTG
CCGGGTCAGAAAAAATGGTGTTGCCGCGCCATCTGCCACCAGCCAGCTATCAACTCGCGCCCTGGAAGGGATTTTTGAAGC
AACTCATCGATTGATTTACGGCGCTAAGGATGACTCTGGTCAGAGATACCTGGCCTGGTCTGGACACAGTGCCCGTGTCGG
AGCCGCGCGAGATATGGCCCGCGCTGGAGTTTCAATACCGGAGATCATGCAAGCTGGTGGCTGGACCAATGTAAATATTGT
CATGAACTATATCCGTAACCTGGATAGTGAAACAGGGGCAATGGTGCGCCTGCTGGAAGATGGCGATTAGccattaacgcg

End of Cre

gggggatctttgtga AGGAACCTTACTTCTGTGGTGTGACATAATTGGACAAACTACCTACAGAGATTTAAAGCTCTAA

SV40 small t intron and poly A

GGTAAATATAAAATTTTTAAGTGTATAATGTGTTAAACTACTGATTCTAATTGTTTGTGTATTTTAGATTCCAACCTATGG
AACTGATGAATGGGAGCAGTGGTGGAATGCCTTTAATGAGGAAAACCTGTTTTGCTCAGAAGAAATGCCATCTAGTGATGA
TGAGGCTACTGCTGACTCTCAACATTCTACTCCTCCAAAAAGAAGAGAAAGGTAGAAGACCCCAAGGACTTTCCTTCAGAA
TTGCTAAGTTTTTTGAGTCATGCTGTGTTTAGTAATAGAACTCTTGCTTGCTTTGCTATTTACACCACAAAGGAAAAAGCT
GCACTGCTATACAAGAAAATTATGGAAAAATATTCTGTAACCTTTATAAGTAGGCATAACAGTTATAATCATAACATACTG
TTTTTTCTTACTCCACACAGGCATAGAGTGTCTGCTATTAATAACTATGCTCAAAAATTGTGTACCTTTAGCTTTTTAATT
TGTAAAGGGGTTAATAAGGAATATTTGATGTATAGTGCCTTGACTAGAGATCATAATCAGCCATACCACATTTGTAGAGGT
TTTACTTGCTTTAAAAAACCTCCCACACCTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTTGTTGTTAACTTGTT
TATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAG
TTGTGGTTTGTCCAAACTCATCAATGTATCTTATCATGTCTGGATC gatccactagatcgaattcc gaagttcctattc

End of SV40 small t intron and poly A

Frt

Tctagaaagtataggaacttc aggtctgaagaggagtttacgtccagcccaagctagcttggctgcaggtcgtggtacga

Start of Kan/Neo

aattctaccgggggaggcgcttttcccaaggcagtctggagcatgcgctttagcagccccgctgggcacttggcgctacac
aagtggcctctggcctcgcacacattccacatccaccggtaggcgccaaccggctccgttctttggtggccccttcgcgcc
accttctactcctcccctagtcaggaagttcccccccgccccgcagctcgcgtcgtgcaggacgtgacaaatggaagtagc
acgtctcactagtctcgtgcagatggacagcaccgctgagcaatggaagcgggtaggcctttggggcagcggccaatagca
gctttgctccttcgctttctgggctcagaggctgggaaggggtgggtccgggggcgggctcaggggcgggctcaggggcgg
ggcgggcgcccgaaggtcctccggaggcccggcattctgcacgcttcaaaagcgcacgtctgccgcgctgttctcctcttc
ctcatctccgggcctttcgacctgcagcctgttgacaattaatcatcggcatagtatatcggcatagtataatacgacaag
gtgaggaactaaaccatgggatcggccattgaacaagatggattgcacgcaggttctccggccgcttgggtggagaggcta
ttcggctatgactgggcacaacagacaatcggctgctctgatgccgccgtgttccggctgtcagcgcaggggcgcccggtt
ctttttgtcaagaccgacctgtccggtgccctgaatgaactgcaggacgaggcagcgcggctatcgtggctggccacgacg
ggcgttccttgcgcagctgtgctcgacgttgtcactgaagcgggaagggactggctgctattgggcgaagtgccggggcag
gatctcctgtcatctcaccttgctcctgccgagaaagtatccatcatggctgatgcaatgcggcggctgcatacgcttgat
ccggctacctgcccattcgaccaccaagcgaaacatcgcatcgagcgagcacgtactcggatggaagccggtcttgtcgat
caggatgatctggacgaagagcatcaggggctcgcgccagccgaactgttcgccaggctcaaggcgcgcatgcccgacggc
gatgatctcgtcgtgacccatggcgatgcctgcttgccgaatatcatggtggaaaatggccgcttttctggattcatcgac
tgtggccggctgggtgtggcggaccgctatcaggacatagcgttggctacccgtgatattgctgaagagcttggcggcgaa

tgggctgaccgcttcctcgtgctttacggtatcgccgctcccgattcgcagcgcatcgccttctatcgccttcttgacgag
ttcttctgaggggatcaattctctagagctcgctgatcagcctcgactgtgccttctagttgccagccatctgttgtttgc
ccctcccccgtgccttccttgaccctggaaggtgccactcccactgtcctttcctaataaaatgaggaaattgcatcgcat
tgtctgagtaggtgtcattctattctgggggggtggggtggggcaggacagcaagggggaggattgggaagacaatagcagg
catgctggggatgcggtgggctctatggcttctgaggcggaaagaaccagctggggctcgactagagcttgcggaacccctt

End of Kan/Neo

c gaagttcctattctctagaaagtataggaacttc atcagtcaggtac CTGGCTAAAGGACTTTCCCTGCGCTCAGTG

Frt P450 continues

CTGGTCAAAGGCTGCCAACCTTTCCTGAGCCCTACGTGGCAGGGTCCAGTGCTGAGTACTGGAAAGGGAGCTGGTACCTCT

ACTAGCAGTCCTAGGTCCTTCAATGAGATCCCTTCCCCTGGCGACAATGGTTGGCTAAACCTGTACCACTTCTGGAGGGAG

P450-R

AGTGGCACACAGAAAATCCATTACCATCAGATGCAGAGTTTCCAAAAGTATGGCCCCATTTACAGgtaagcctggcagcag

Exon/intron junction

gtggagcaggcacaggggaggcacacggctcctgcttcctgtctgccttttttctttcatttcttccaggctggggctggg
gctggggctggggctggggctggggctggggctggggctggggctggggctggggctggggctggggctggggctggggct
ggggctggggctggggctgaggggatagtcttccctcatttctgactccaagacttttttctactggtataaagaagtgtga
gcaagcagtgcccccctcacccaccaactttagccaccctaaaagtcaagtttgctgtctctatcttcaggggaaggaaa
tgagacattggtgccctgactactggcaagccccgaccaggctttagcgccagcctcagcttcaggccctttgtgtctggg
agctgagcaagaacagtacacgctcttaatcactgagccatctccccaccttgacatttttctttaatagaagtgaagggt
gccatctcacttttgaggcgagtgactactctgaattacaattcaagaagagccgggctggcttggaacttgggagataca
gggcattcactgtagaatgacaagaaagcctgccatggcttcaagcccagcaggacaagaaggttcagcgtggaatcggca
tgattatgaaatggaaggattggggtgggagtggggctggtcctggggtgcgctgtgtggtgtatgatagtgtcctgccgt
cctttggaatgagtcacaatgtcataggaaaatgtgtcatgtttggctcttcagcagctccgcaagacagatgtcacatcc
atttcccatctcataggtgacactgaggctcagcaaagggaaggtcccagccagaaagctgtgtactgattgtgtagagag
gcctggctgcccagggtgtgtgttcacggctgcctcaggtccaacatcagccgctcttgtctttccagggagaagctgggc
actttggagtcagtttacatcgtggaccccaaggatgcgtcgatactcttctcatgcgagggtcccaacccggagcggttc
cttgtgccccctgggtggcctatcaccagtattatcagaggcccattggggtcctgtttaagtgagttctgagtgagcct
cgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtagggtgctgaaggaaagtggtagggagaggggtc
gggggaaagagtggctctgctcatcttcctgttccataaggcaaacgatggtgacaacagccatgacagggaagtgtcctg
ccgactcccaggggatgtcaggctgaagttcttcatgaatagtggagactttgcaaagttctcactaattgctttaacta
tgaagtgccactttatcaaacacctgctaggtaccaggccttgtgccaggtgttggcagtacagctgtaaataagggcctg
acagcctgaagccactgtgagaaacagcgagatctgccactgagatatagatgctattttctaccaaattgcatactgata
aatttatatatatttatatataatttttatataaatatataatttatatataaatataaataacatataaaatatctatct
atctatgtatctgtctatgtttctacattgatagggtctttctacattatctagaactttctagaagttgctacatagacc
aggctggcctcaaacaaacatcagcttgctttggcctcctttaaaggttaaggacatgtgccaccacacccagtgtcactg
aaacttttaaatgtgactaaatcaagggttggcaaggccctggagcaactggagctctcgtgcactgtggtgggaacatgt
acctggcctctgtgtgaacagtgcttggatgttaactcccaagaccgactattaccagggccacggtggctgtgagtcaca
cagtgtttctaactttagtgacatgccatgtcctctcagggaacagggtcataggctagaatgtttctccaggctatagtt
aaaaaaaaaagaaagaaaaaagaaggaaagaaaaagaaaaaccaggaagccttctagctggttgagatgaaggaggacggt
ggacgagagaaggttgcatatacctgccacaatgtacaggtacagactcaggtccacgacacggctgtcctgaaatgcaaa
actgagtggagaaggcaagtactccaaactctgacaggcatttagaaactctggagctagcacagtctttttagtgtcttt
taagagagattcagtaaaaccatacacaaaaggggaagggacccaggctcgggtgggggcttcatgtggagcagggtag
gctgaaaccatgagtttacaaaattatttgcctccagactctcaacttttaaaagaagttttattcatagaattctatt
tgtgtgtatatgtctggtaagtatatgccatgtgtatacagatgcctctggggactaggagagggcaccggatcccctgtg
gttgttacaagtggtccagagttgccctatagggatgctgggaactggacttgattcctctctaagagcagtgagccatct
tgccagctcacaacgttcttaaacaaacaaactcccccaaattacaaaaggagcattgcagccgggcagtggtggtgcaca
cctctaatcccagcacttgggaggcaggggcaggcagatctctgagtctgagaccaggcagggcagggagggagtaggaga
ggagagcaaatgttgggaggaggggagatgatgttgctgcaggtgctggagctagagagcctagcaagtccgtaggtgcca
ggtgtggcatctgtgaccttgaggcgggacctgtgtttctctaggagttcagatgcctggaagaaagaccgaatcgtccta
aaccaagaggtgatggcgcctggagccatcaagaacttcgtgcccctgctggaaggtgtagctcaggacttcatcaaagtc
ttacacagacgcatcaagcagcaaaattctggaaatttctcaggggtcatcagtgatgacctattccgcttttcctttgag
tgtaaggatcttacacatgactgactggcaggagctgggggtggggagccagcgttcacccttttaccttgagaccccagg
ccctgtgcttgtgtggattggtttcttggacagactctgtactgtcagtctctcgggggtagtcaaatagctctcatctga
cattcatgttggggaagtccccagatcataggttttccatgccaccatcctccataagccagaacttggcttggccctgca

tgctgcaagtctccctctcgtagaaacagacctctggagttgagcaagaaagaggggacctcctgggttgatctgggctga
ctgttgaaggacacttagaaattatccagactgggtaaggcggttaggcgtcatagaccaggcatagaacagtgcatgcat
atggcttagtagaagtcatgtgatgcctgcaggagatctggggtaccctgggcttggtataaaattggcattcaaattttt
cttgtggtaagtgagaaaacttgaatcttttagtttcagccagattgaatcctgggtgacttggagactgctaacctaagt
ctcccaggccatgtgtgatgtagtgattgcagtcagtaacaaagggaaatctctcctggacagttccgtcctggctcaggt
ggagtccactattcccatcaaagcgtcctgatcagggccgcccctatatccctctgagggacctgaattgaagccagaagg
gcagtcttcttccatctcttggggctcaggtctcagttgctttgctggaggaagctaaggatgagcaagggcccctgtgtg
tggtgggcatggatggggatggttctgggtgctgaggctcttggagagccaggctggaagagggtccttagcctcagtctc
tc

c. 5’ Probe

cttgacactgcttcctgagttgagttttgttatgatggggctgataacctcccccgaccctgaccccaacccaatgaatttga
atgaaaagctcagctctgaagtctttgtagtattcatccatccttaggtcttctaggcctctactacagtgactggatcagagttgttg
taaagactcattttgcactcttgtgaaactttcaggaagggctgttaccggagaaggagggcggagctgatttgcatgtcctgcctctc
cggcttggagggagggatgggttgagagctatccaggatgtgcttgctctaagatcagttcctctccctattggtttcttcagaggcca
accattcttggggctgaggcgggaaatggtttcaggtgagatcaggtgcgaaggttggaaatgcagcaagcctgcacaaaccgttgctt
ccttcccaagt

d. 3’ Probe

actgcagccatcagcagtgttatatttggggagcgcatggggatgctggaggagatcgtggatcccgaggcccagcggttcat
caatgctgtctaccagatgttccacaccagtgtccccatgctcaacctgcctccagacttctttcgactcctcagaactaagacctgga
aggaccatgcagctgcctgggatgtgattttcaataaaggtgaggactcctctggcagtactcatgcctgaccaatggccgcctctgtt
ccacaattaaccaatggccactaatctaccatggagggaaatctcagatgaacaaggatgtgtgtcagggctggaggctgactattgga
gttggttacttttccttctggagagagggaataaggatgttcatggtactcatgacga