NIH-0683 Genotyping Strategies

Reaction Components	Vol (ul)
10X Sigma Buffer	5
25mM MgCl2	3.5
10mM dNTPs	2
Primer 20 uM	1.5
Primer 20 uM	1.5
5 U/ul Taq polymerase	0.5
Water	31
Total mix volume	45
Tail lysate (1:20 dilution)	5
Total reaction volume	50

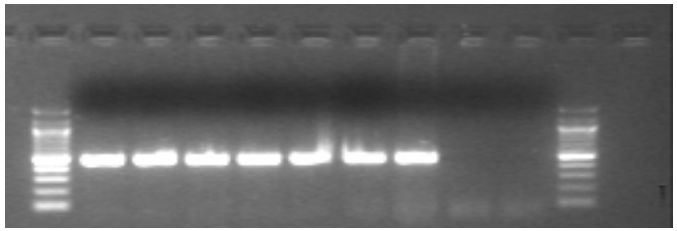
Step	Temp	Time	Note
1	94C	15"	
2	65C	30"	Decrease 1C/cycle
3	72C	40"	Go to 1, 10 cycles
4	94C	15"	
5	55C	30"	
6	72C	40"	Go to 4, 30 cycles

Primer Sequences (5' to 3'):	
Mutant PCR: Primer Neo3A and Primer 0683-7, 471 bp	
Recommended Wt PCR: Primer 0683-1 and Primer 0683-7, 408 bp	
Primer Neo3A	GCAGCGCATCGCCTTCTATC
Primer 0683-7	GAAATCTGGAATCTCGGAGC
Primer 0683-1	AGCTTTTGACTAATACCGTGC

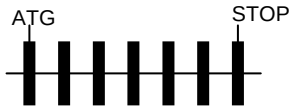
Well	Sample	Genotype
1	10	het
2	19	het
3	27	het
4	79	het
5	85	het
6	95	het
7	ES DNA	het
8	wt lysate	wt
9	water	no amp

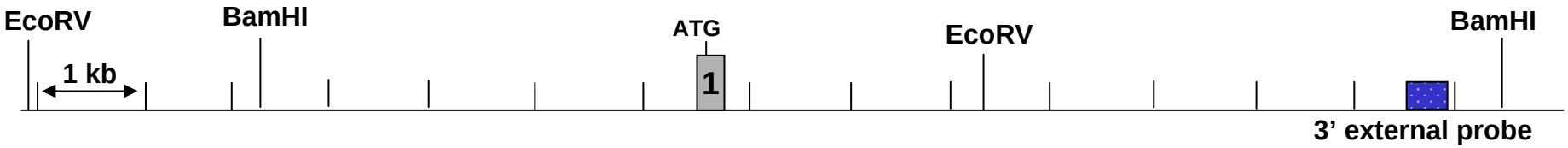
Mutant PCR

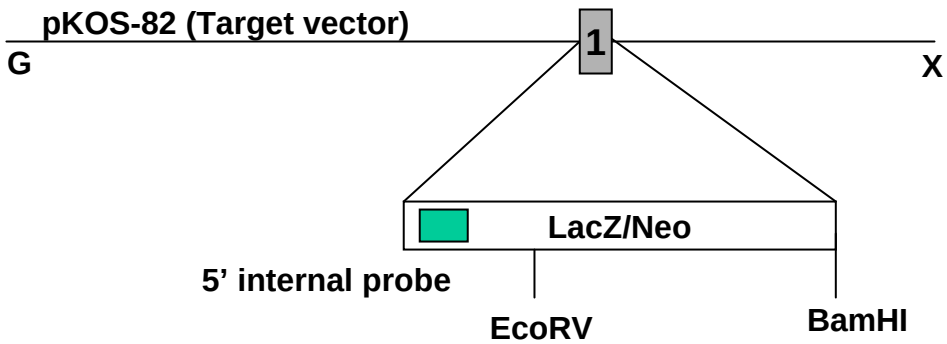
MK 1 2 3 4 5 6 7 8 9 MK



Targeting Strategy



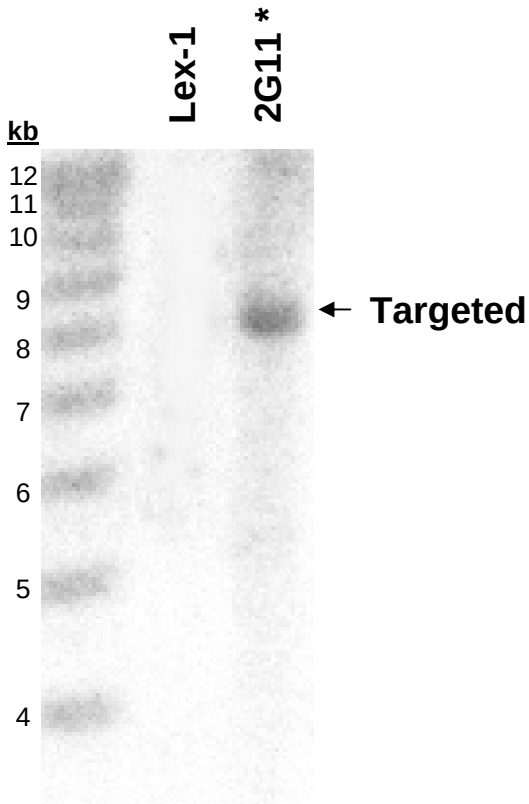




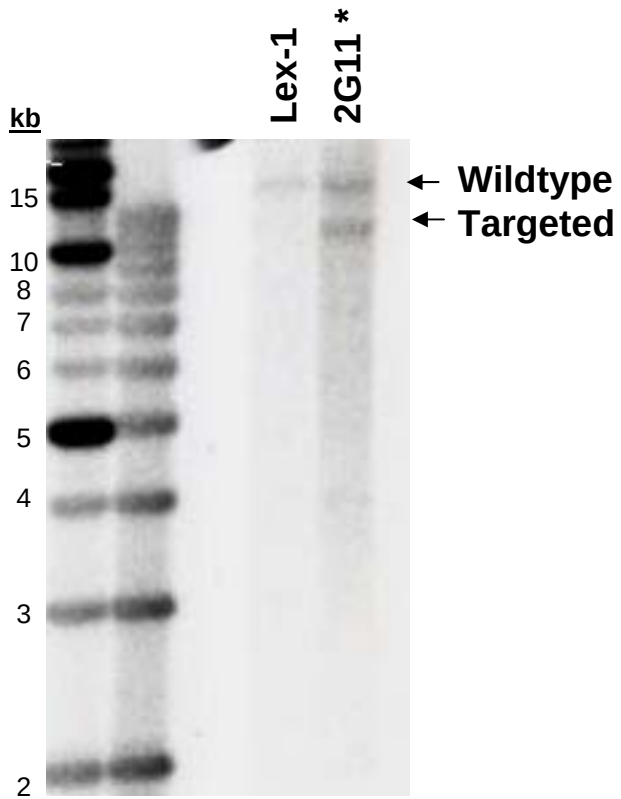
Southern Strategies

Probe	5’ internal	3’ external
Enzyme	EcoRV	BamHI
Wildtype	----------	15.4 kb
Targeted	8.1 kb	11.2 kb

Southern Data



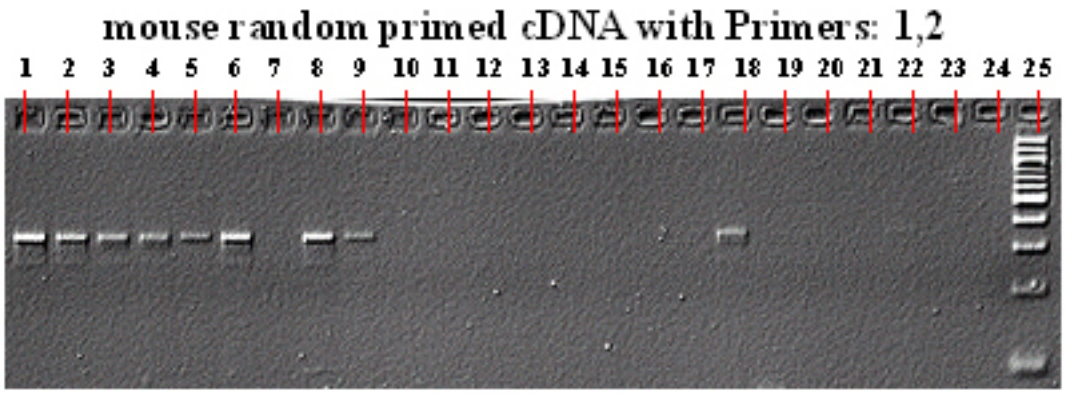
5' internal probe
EcoRV digests
Targeted: 8.1



3' external probe
BamHI digests
Wildtype: 15.4 kb
Targeted: 11.2 kb

*** Clone achieving germline transmission**

RT-PCR WT Expression Analysis



02/21/2003

Note: Expected band size denoted by arrow adjacent to 100bp ladder/marker.

Mouse cDNA Tissues

1) Brain
2) Spinal Cord
3) Eye
4) Thymus
5) Spleen
6) Lung
7) *Blank-TBD*
8) Kidney
9) Liver
10) *Blank-TBD*
11) Skeletal Muscle
12) Bone
13) Stomach, Small Intestine & Colon
14) Adipose
15) Internal test
16) Internal test
17) Internal test
18) Heart
19) *Blank-TBD*
20) Internal test
21) Blank-TBD
22) (+) Control- ES cell cDNA
23) (-) Control
24) (+) Control- Genomic/Lex1 DNA
25) 100 bp ladder/marker

Lexicon Genetics Incorporated
Molecular Genetics Project Materials

Catalog Number: **NIH-0683** (LEXKO-403)

Reference accession(s): NM_153581

Standard KO or Conditional: Standard

Materials Submitted: ☒ Target Vector pKOS-82TVneo
☒KOS clone(s) pKOS-82

Southern Blot Genotyping Strategies:

	5' Internal	**3' External**
Name of Probe:	Internal 8/9	51/52
Restriction Enzyme for Genomic Digest:	EcoRV	BamHI
Predicted Wild-type Band (kb):	-----	15.4 kb
Predicted Mutant Band (kb):	8.1 kb	11.2 kb
Probe Size:	291 bp	478 bp

Primer sequences:

Southern probes

0683-51 5' – GACATTGCACACCGTATTCC
0683-52 5' – TTCTTCCCAGAATAGAAAGGG
Internal 8 5' – AGGAAGCAGTTCCTCTGGAA
Internal 9 5' –CACATGTAAAGCATGTGCACC

Genomic Sequence Deleted:

CTTACTGTGGCACACACAATGGGCAGCTTTGAGAAGAAAAATTGATAATCTTCACGGAAGAGTAATTTGAATGAAATTACACTTGACAGCCAGTCTCT
AAGCAAACAAGAGGCGCGAGGGAGCCTCAGCTAAGCTCCAAGGACTTGCCCAAGCCACTGCTGCTGGAGCTGCCCAGGGAAAAAAGGCACACGCTTT
TTTGACATCTAATTGAGCTTTTGACTAATACCGTGCGCCAGATTCTCCTGAAGAAAGGTAGCCATGGAAGAGAATATGGAAGAAGGACAGACACAGAA
AGGTACTGTGCT

KOS clone sequence: *(note: pKOS-82 was used to generate the TV and that is the sequence included here)*

GATCACCAAAGGATGCAGGACTGGAACTCAAGCAGGTCAGAAAGCAGGAGCTGATGCACAAGCCATGGAGGGATGTTCCTTACTGGCTTGATTCCCCT
GGCTTGCTCAGCCTACCCTCTCATAGAACCAAGACTACCAGCCCAGAGAGGTCCCACCCACAAGGGGCCTTTCCCCGTGATCACTAATTGAGAAAATT
CCTTACAGTTGGATCTCATGGAGGCATTTCCTCAACTGAAGCTCCTTTCTCTGTGATAACTCCAGCTATGTCAAGTTGACACAAAACTAGCCAGTACAT
ATCTCTTGTATTTTGTTGGTGATTCTTGCCTCTTTGACTCCTGATCTCTTTCTCAGGTTTTCTATCTCCAGGCTTGTCTCCCTTTGTGATTTCTTTAATGTTT
CTATTTCCATTTTTTTGATTATGAAAGGTTTTGTTCAAGTCCTTCACCTGTTTGTTTTTGTTTTCCTATAATTCTTTAAGGGATTTTTGTGTTTCCTCTTTAA
AGGCTTCTAGCTGTTTACCTATGTTCTCCTGTATTTCTTTCGGGGAGTTATTTATATCCTTATTAGAGTCCGCTATCTGCATCATGAGATGTGATTTTTAA
ATTCAAATCTTGCTTTTCCAGTGTGTTGGGGTATCCAGTATTTGCTGTTGTGGGAGAACTGGGTTCTGATGATTTCTTGTAGCCTTGGTTTTTGTTGGTAA
TGTTCTTGTGCTTGCCTTTTGCCATCTGGTTATCTTTGGTGGTGGTATTAGCTGGTCTTGCTGTCTTTGACTGTGGGTTGTCCCTCCTGCAAGCCTATGTGT
TGGTACTCTTGTCAGACCAGCTCTCTCCCAGGGGTATTTGGGTATGGAGAGCTGGGTCACAGGATCAGCTCCCAGTACAGACTAAAACTGGAAGGATC
CTGTCCCGGGCTACTCCTTGGATCCTATGTCCTGAGGGTTCTGGGTAGGTCACTCAGAGCAGAATTGGTGGTCTTACCTGTGTTCACAGGCATGTCTGCA
CTCCTGGGAGACCAGCTCTCACCAGAGAGTTTGGGTATTGAGTGCTGTCTTAAATTTTCAACCTCTACTCTAAGGCAGTAGTCAATTTGTGTTTTATTTC
TTTTTCTAAATATAGCAAAAGTGGCAATCTAAATATTTCATTCATGAGATGATGCTTTGTCTAACTTTGAAAGATTTCAAGATAAAAAGAAAATTTTAA
ATTGGTCATGACTCTTGGTTCTATACACTATACTTAGTCCACACAATCATATTTTTCACATGTCCTATTTACTCTATGACTCAGAACCCATAAAAAGTTTC
TGTCATTTTTCTTATATTCTTTCTACATATGCTTTGTAGTAACTAATAGGTCATAATTCTAGTTGTTTCTAAGAATACAGTTGAATATAAAGATAAGTACA
TTATACAGTTGAGGGATATGTAGATATTTTCTCAATTGATAGCATGTCTTAAAATGGAGATGTATTTAAACCAAAGTTAGGAAAAATGAGAAGGACTTA
CAATTTTAGACTGTGTAAGTAAGCTATCTTTTAGTCCACATGGGAAAAATAATATACATCATTAATACATCTCAAATATTATTTCAAAGAATAATCTTCT
TTAACATTAGTGGTAATAGTTTTTAGGTGTCCATTTATGTATTTAAAACACCACAAGTAGAACTAGAGTCAGGGCAAATACAATGAAAATAATTGAAA
AAAATATTTGATGAGGCACTATATCACCATAACTTGTGTATTTTAATGATCATTGTAGTTACTTTTCTATTGCTTTGTTAAAACACCTTGGCTAAAGCAA
GTTATGAAAGAAAATGTTTAATTTGAGGCATTTGGTTTGAGAATGTTAGAGGTCACACCAATCATGGTGGGGGGCTGTGGCAGCATACATGCGGGCATT
GTCCGGAGTGGTAACTGAGAACTTACATCTTGATAACCAGGCATGAGTCTAGAAGAGCTAACAGGGAATGGTGTGGGCTTTTGAGCTTTCACAGTCTTC
CCTCAATAACATAACTTCACCAACAAGGCCACACCTCTAATCCTCCTTAAAGAGTTCCACCAATTTGGGACCAAGATTTCAAATATATAATCCTACTGA
CACCATTCTCATTCAAACATACTACAATCGAAATACAGAGTGAAGATCATTGGCAGTTACTAAGGTTAATGTTTTATATTTAGAGTTCATTAAAATCAG
TTTTCTATAAGGTTTTCTCATGAACATCTCATGAACTACTTTTCACATAACTAGATATTTAAGTTTTTTATTCAAACGACAAAAGATTTTGCAAATATCCT
AAAAATGTCAACCAGCAACATCAATCCCAGCTGTCAAATGGGAAAAGAAAGATGGGGAATTTGGGAGATTTAAAGTGTTTCAGAGTGGGGTAGTGGA
AACAATGCTAGATAAATGTGAATATACTGAAAACCCACAGGAGTGTAGACTTCAAAATGGCTCATATTTGTGTATATATCAAAAAAATGAATCTTAGC
TTGTTTACAGGTTGTTGATGTAAGAATATGTATGTATGCGTTTCTTCTCATCATTTTGGAAGCTCTCCATTATCTTCAATATGAAAGGATGATGCTCTGA
ATATTTTAAAACCAAGGACGGTTTGGGAAATGAACTTCTTAATTAGTAAAGGAATCGGACATGGGAATGGGAAATAATGGGGACATCTGACACAAAAT
AACCACACAAATATCTATTTTCCACCTAGAGTTGTGAATACTGTAGAGTGGATGGTGTGGGAAACATCTAGGATGTTGGGGAACGGGGCGTCAACAGA
ACACTGTGTCTTGAGAATGAAGCACTTCACTTGCATATGACCTATAAACAAATTATTTCCTTAAAAGGGATTCTTATCAGAAAAAATAAAATAAAACAA
ATAATGAGTTGCTTTTGTTTTATAAAGAAAACCATTCTGACAATAAGTTGGTTATTATACAAATATATTGTTCCCTTGAAGTTTCCAGAGTACAAAAGA
AGTTGTAATCACATCTTTCTATTGATCTTGTCCAGCAAATATTGTAAACTGAGGGTGACAGTGAAGGGACCAGTGATGTATTGGAGTAGAAAGGTAGA
AGAGTTTTAGAAGTCAGAGTCCTAGACAAAGAATCAGCTTATGATGTTTGCTCTAAGAGTTTCTTCTTCATTTACAATCCCAGACCCTGCATATGCTTGA
GTAGCAGACGAATATTCCAACATACAGGCTAATATATATATATATATATATATATATATATATATATATATATATATATATATTTCCCTGTTTTCCTTAAGTAA
CTCACTTAAAGTTTATAAAATACCAAAAAATATAATATAGTGTTATCATTTGTGTGTGTGCTGCTGTTAATTATTTGCTACACTGATTATTTCTGTGTCGT
AAGATGAATGAAGCAGGTAAACAAGGATGGAGGCTCCACTCTGTTCTGAGTGTATGCCAGGATATGTCAGCAGAGAGATGGGCTGAAACACAAAGAC
TGCTATGTTTTGGCTTCTTTGATTGCCCTATGGCTTCTCTTTCAATCTGGACTGAACAAGCAACTGTCTTAAAAATCCTTGAATACAAATATATATATAT
GGGGAAAGAGACGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGGAAAGGGAGATAGATGCTGGGTGGCTAGAGATTCTTCAGT
CGCAGTTACCCTTGGGTTAAACACATTTGACGAAAAGAATAGAAATATTGATTATCAGAGAGAAGTAGCTCTTCATTTATGTAAATAAACTGGAGCATT
GTCAGATCATTTTTCCTTGAAATATATAAGTGATTTTACAGTTAAAAGTGTGGTGTGAAAAAATGCTGCTGACATGTCTTACAACCTATGTGAAAATTTT
CGTATAACGCAAGCATATTATATGGATAACGGGGATATGCATTTTTTTTTCCATCAAGGGCAAGAGCTTATTCAGCTCTGTGTGTTTCCATTGCATGTTT
GTTTCGAGAGGAACATAGGCAAGAGAGTCTTCCTTTATACTCTAGGAAAGCTTGACACAGCCTTGACAGACAGTGTTTATGTTCTTCCTGTACCTTTTGT
GAAGTATTGCGACTCTACTTGTGTCCTGTCACTCAATAGGTTGAGCACGACTGCTAATTTATTGCCAGACTGCAGCATTGTGATTTGCATTCCTAATCAG
AGTGCAGATTCCCATTGTAAAATGCTATCCTGTTGTTCCCTATCCAGAATCCTTTCGTTCTTTTTGTCGCCCCCATTCTCCAATAATCACCCTTAGAATTT
TTCCCTTCACAAAATTCCCACTCGGAATTGGCAGCTTTTGTGGGCTAAACTAATCATAATTTACAAAGACATCGATTCTTTTTCTCTTTACCTGTAAAGG
TCCTACGCCACTTAATAAAATACTTATTACAACAAAAGATTTATACTTATTTCTTTGAAAACCATGTTTTTATTTCTCCTTGATTCTGAAAGACGTGAAA
TAAAGGTCCTATCTTAATAATAAGCTTTAACAGACCAGCCTCGAGATAGTCTAATTACATCCCTCCTTGGCACCTTTAAATCAAATTAACTAGACACTT
GGTGAGGAGGTAAAAGGAAAGGGCATTTTTGTAATGAATCTGACTGGGTGAGAAAAAACTACTGCAGCAGAAAGACACGACGAGAATTCGGGCTTTC
CCACTGTGGTGGCAAAGGGGGGAATTATGGGTTGGTGGTCCGCTTCATGCTAGGAGGACACCCCTCTGTCTGTTCACAGTTCAGCCTGCTTCCAGTTTA
AAGCCGAGATTTCAGCACAAACCTCAATTTCTTTCCCCCATTTTAAATGCAAAGCGAGGCTTGTGAATCACAGTGTCTCTGCTCCTAGAATCCAGACCC
CTTTCCACTCCCCCTCCCAAAATTATCCGGCTGTTTGTTTGAGTACCTGCTTACTGTGGCACACACAATGGGCAGCTTTGAGAAGAAAAATTGATAATCT
TCACGGAAGAGTAATTTGAATGAAATTACACTTGACAGCCAGTCTCTAAGCAAACAAGAGGCGCGAGGGAGCCTCAGCTAAGCTCCAAGGACTTGCCC
AAGCCACTGCTGCTGGAGCTGCCCAGGGAAAAAAGGCACACGCTTTTTTGACATCTAATTGAGCTTTTGACTAATACCGTGCGCCAGATTCTCCTGAAG
AAAGGTAGCCATGGAAGAGAATATGGAAGAAGGACAGACACAGAAAGGTACTGTGCTTTAAAACAGGTTTATTTGTAACTAGGTGATTATTTTGTGCT
TTCCTTGTTTGCTTTAGACACTTGCCAGAGAGACTAATGAATCTTTCCCTCTTGGAAGGCATTTCTCCCTTCCTTTTTCCTCTGGTCTTCACTTGCTGTAAT
GCCAATTGTGATCCTTACTATTTGAACATCTTCTAGGTATAATACCTAAACGCATATTCTGATATTGATATGTACAGTGTGTCGAACTAATCTTTAGTCC
AGTCTTTGTTAGACTTCATTTATTTCATGTTTTCATGGTTGTCAAGCCAAGCTCCGAGATTCCAGATTTCAGGTACAAACACAGTTGGTGCACAAGTTGC
AGTTAAAATTTTGATGATGTAACCATTATCAATACCTTTGCATAGCCATCTGATAGAACAAAGAGCAACAGCATGTAGTATAATATGGTTGGTATTCAT
TGAATTTATTTATTTTTCTGGATGCAGTGTTGTTTTAATTTATAAGGAGCAAAGGTTCACTGATTTGGGGGGGTTATTTAATGACATTAGATTTTTTTTTC
GTATGTCTGATTATAATCAACCTATACCTATATCATTGTTAAATATCAGATTCATTTTTAAAGCTTTCTAGTTATATCTGTCTATATGTGGCAATTTTTTA
AATTAAAATTGGACAAATGCTGCCCATTTCAAATGATTCAAACAGCTTTAGAATTTTTTTTTATACATCAAGTGGGGCTTCCGAGGAAATTGTTTTCACC
CCAGTAGAGGCAGTGTTTGCTGTCATGTCTGTGCACTGGCTTATGTAACATTAGAGAAAACACTCATTTTCTTAAGATTAAAAGACTCATAGCACGTAA
TACACATAAAGAAATTAATTTATGAGGATTACTAAAAAGAGATGTTTTCAAGGATGATTTTAAATGCATATAAATTTGTTCTCGTTTTAAACCTGCGTTT
TTAATGATGTCACTGGTAGTGTGGAGATTCAGTGTAGGCTTTCTCATGTTAAAATGTTCAGGTATGCTTGCCCTCTTCTCTAAGTATATACACACACACA
CCATACACACACATACACACACAGAAGCATACACACACACATACATATGTGTACACACAGGCAGATACACACACACCGAAACCCACACACCCCTTTGG

GTAAACATGCTGTACACAGATCCACCTGTATACAACGACTAAGTATGTAAATGTTTTCTATATGCAATAAAGATAAAAGATAAGCTAATAATCTTCAAA
GCTTATGCTCTCATTGATAATGGCATTTGAAGAGAAACATAAAAATCAGCATCACTATAATACTAAGCTTTTAAAATAAAAACACCAACATTATTTGTC
ATTGCACAAATAACTTAGGAGTTTATTAGAGATTTTAAAGGGTAAATTTCCCTACCATTTCCTGTCTATACTACACTAGACATTTCAGGGGACTGCTTTA
TTTTCTGAATAAATCTCTTATTCTCACATCTTTTGATTGTACAAGTCAAATATTCTTCTTAAGCAAAGAAAAGAGTAGTTCAGTCATTTTAAGTAGACAA
ATGACACTCATTACACTAAATAAAATCTTAAATTATGGACCATGGTAACAGGTTGAATGTTTCTCAGCAAGCTATGATAGAGGCAAGAACAAGAAAAA
TGATGTAATGATGTTATGATCTCCCTCCCTCCTCGGGCAATACTAAAAATCGTTCATAGAGGGGAGGCAGCAGAACATTGACAGGATGATCTGTCTCAG
TGGGTGTCTTATCATTAACATATGTCCATATAGACCAGCAATAGTGGAGGAAAGCGATGTTAAAATTAAAACAATCTATATGGTATACAATAAGTACA
TGATGGTTCTATGTAAAATATCTGTGGATCGGGTTGTTTTCTTAACTGAGGAATGGATTTAATTCTCTTTATACCTGAGTATCCTTTTCTCCCCCTCAAAT
ATACCCATGGCACTTTTTTAACATGTTGGATTTTGTCAAGTTACCTTTGAACAGAAATAGAGAGGAAACATTGTGAATTTTGTATTGATTGCTTTTAGAG
GAATTATTTGATAAGCAGATCAAATCATTGGAAATGTTTTAAGGGTTCAGGTAGACAGATTTAATGATTCATCTCCTGGAAAGGAAAGCAGCATTTTAT
ATTCTCTCCTCTCTTGTTTTCTTAAATCACCCAGGATTTTCGATACAGATTCTGATACCAGCATACTCTCTTGATTGAAAGGCATTTCCCTGTCTTCTATT
AATTTAACATCTTTTATTGTACTTAATGTTATTGCTTCTGTTTAAACCGAAGACAACCAGATTATATTAAACAGATAAGCATTACGCCAAATGAACACTT
GAATTCAATTGTAGAGTGTGCTAACAGGTTAAATGCTCTGGGTGAGCCATAGCAGATGCAGCAACCAAGGAAAGTGATGTCATTGTTTGTTATCCATTT
TTCCAGCAGTAACTTTGAAAAATTATTCTCTCTCAGAGGAAGTGGCAACAAATACGTTTTCTTTGCTATAATTTAGACAGATTGAAATTTGTATCACCAT
CTACCCATACAACCTTTTAAATTCCATGAGAATGTAAAATTTAATCCACTACTTTAACTGAATATATCAGAGAAGGATAGTTTGTTGATGGTTAAGGAC
TTAAAAGGGTCTCCATAAGAAATGTGTTTTTCTTTGTATTTATGTGTCCTTCCCCACATTGTCTGTAAAAGGCAAACAGAAAAGGTGATATCCAGATGC
ATATTCAGATC

Selection cassette sequence: *(note: linker sequences may vary and are not provided)*

LacZ/Neo

GGCGCGCCGGATCCCGGGCCGCTCTAGCTAGACTAGTCTAGCTAGAGAATTCCGCCCCCCCCCCCCCCCCCCTCTCCCTCCCCCCCCCCTAACGTTACT
GGCCGAAGCCGCTTGGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATTTTCCACCATATTGCCGTCTTTTGGCAATGTGAGGGCCCGGAAACCTGGC
CCTGTCTTCTTGACGAGCATTCCTAGGGGTCTTTCCCCTCTCGCCAAAGGAATGCAAGGTCTGTTGAATGTCGTGAAGGAAGCAGTTCCTCTGGAAGCT
TCTTGAAGACAAACAACGTCTGTAGCGACCCTTTGCAGGCAGCGGAACCCCCCACCTGGCGACAGGTGCCTCTGCGGCCAAAAGCCACGTGTATAAGA
TACACCTGCAAAGGCGGCACAACCCCAGTGCCACGTTGTGAGTTGGATAGTTGTGGAAAGAGTCAAATGGCTCTCCTCAAGCGTATTCAACAAGGGGC
TGAAGGATGCCCAGAAGGTACCCCATTGTATGGGATCTGATCTGGGGCCTCGGTGCACATGCTTTACATGTGTTTAGTCGAGGTTAAAAAAACGTCTAG
GCCCCCCGAACCACGGGGACGTGGTTTTCCTTTGAAAAACACGATGATAAGCTTGCCACAACCATGGAAGATCCCGTCGTTTTACAACGTCGTGACTG
GGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCA
ACAGTTGCGCAGCCTGAATGGCGAATGGCGCTTTGCCTGGTTTCCGGCACCAGAAGCGGTGCCGGAAAGCTGGCTGGAGTGCGATCTTCCTGAGGCCG
ATACTGTCGTCGTCCCCTCAAACTGGCAGATGCACGGTTACGATGCGCCCATCTACACCAACGTGACCTATCCCATTACGGTCAATCCGCCGTTTGTTC
CCACGGAGAATCCGACGGGTTGTTACTCGCTCACATTTAATGTTGATGAAAGCTGGCTACAGGAAGGCCAGACGCGAATTATTTTTGATGGCGTTAACT
CGGCGTTTCATCTGTGGTGCAACGGGCGCTGGGTCGGTTACGGCCAGGACAGTCGTTTGCCGTCTGAATTTGACCTGAGCGCATTTTTACGCGCCGGAG
AAAACCGCCTCGCGGTGATGGTGCTGCGCTGGAGTGACGGCAGTTATCTGGAAGATCAGGATATGTGGCGGATGAGCGGCATTTTCCGTGACGTCTCG
TTGCTGCATAAACCGACTACACAAATCAGCGATTTCCATGTTGCCACTCGCTTTAATGATGATTTCAGCCGCGCTGTACTGGAGGCTGAAGTTCAGATG
TGCGGCGAGTTGCGTGACTACCTACGGGTAACAGTTTCTTTATGGCAGGGTGAAACGCAGGTCGCCAGCGGCACCGCGCCTTTCGGCGGTGAAATTAT
CGATGAGCGTGGTGGTTATGCCGATCGCGTCACACTACGTCTGAACGTCGAAAACCCGAAACTGTGGAGCGCCGAAATCCCGAATCTCTATCGTGCGG
TGGTTGAACTGCACACCGCCGACGGCACGCTGATTGAAGCAGAAGCCTGCGATGTCGGTTTCCGCGAGGTGCGGATTGAAAATGGTCTGCTGCTGCTG
AACGGCAAGCCGTTGCTGATTCGAGGCGTTAACCGTCACGAGCATCATCCTCTGCATGGTCAGGTCATGGATGAGCAGACGATGGTGCAGGATATCCT
GCTGATGAAGCAGAACAACTTTAACGCCGTGCGCTGTTCGCATTATCCGAACCATCCGCTGTGGTACACGCTGTGCGACCGCTACGGCCTGTATGTGGT
GGATGAAGCCAATATTGAAACCCACGGCATGGTGCCAATGAATCGTCTGACCGATGATCCGCGCTGGCTACCGGCGATGAGCGAACGCGTAACGCGA
ATGGTGCAGCGCGATCGTAATCACCCGAGTGTGATCATCTGGTCGCTGGGGAATGAATCAGGCCACGGCGCTAATCACGACGCGCTGTATCGCTGGAT
CAAATCTGTCGATCCTTCCCGCCCGGTGCAGTATGAAGGCGGCGGAGCCGACACCACGGCCACCGATATTATTTGCCCGATGTACGCGCGCGTGGATG
AAGACCAGCCCTTCCCGGCTGTGCCGAAATGGTCCATCAAAAAATGGCTTTCGCTACCTGGAGAGACGCGCCCGCTGATCCTTTGCGAATACGCCCAC
GCGATGGGTAACAGTCTTGGCGGTTTCGCTAAATACTGGCAGGCGTTTCGTCAGTATCCCCGTTTACAGGGCGGCTTCGTCTGGGACTGGGTGGATCAG
TCGCTGATTAAATATGATGAAAACGGCAACCCGTGGTCGGCTTACGGCGGTGATTTTGGCGATACGCCGAACGATCGCCAGTTCTGTATGAACGGTCTG
GTCTTTGCCGACCGCACGCCGCATCCAGCGCTGACGGAAGCAAAACACCAGCAGCAGTTTTTCCAGTTCCGTTTATCCGGGCAAACCATCGAAGTGAC
CAGCGAATACCTGTTCCGTCATAGCGATAACGAGCTCCTGCACTGGATGGTGGCGCTGGATGGTAAGCCGCTGGCAAGCGGTGAAGTGCCTCTGGATG
TCGCTCCACAAGGTAAACAGTTGATTGAACTGCCTGAACTACCGCAGCCGGAGAGCGCCGGGCAACTCTGGCTCACAGTACGCGTAGTGCAACCGAAC
GCGACCGCATGGTCAGAAGCCGGGCACATCAGCGCCTGGCAGCAGTGGCGTCTGGCGGAAAACCTCAGTGTGACGCTCCCCGCCGCGTCCCACGCCAT
CCCGCATCTGACCACCAGCGAAATGGATTTTTGCATCGAGCTGGGTAATAAGCGTTGGCAATTTAACCGCCAGTCAGGCTTTCTTTCACAGATGTGGAT
TGGCGATAAAAAACAACTGCTGACGCCGCTGCGCGATCAGTTCACCCGTGCACCGCTGGATAACGACATTGGCGTAAGTGAAGCGACCCGCATTGACC
CTAACGCCTGGGTCGAACGCTGGAAGGCGGCGGGCCATTACCAGGCCGAAGCAGCGTTGTTGCAGTGCACGGCAGATACACTTGCTGATGCGGTGCTG
ATTACGACCGCTCACGCGTGGCAGCATCAGGGGAAAACCTTATTTATCAGCCGGAAAACCTACCGGATTGATGGTAGTGGTCAAATGGCGATTACCGT
TGATGTTGAAGTGGCGAGCGATACACCGCATCCGGCGCGGATTGGCCTGAACTGCCAGCTGGCGCAGGTAGCAGAGCGGGTAAACTGGCTCGGATTAG
GGCCGCAAGAAAACTATCCCGACCGCCTTACTGCCGCCTGTTTTGACCGCTGGGATCTGCCATTGTCAGACATGTATACCCCGTACGTCTTCCCGAGCG
AAAACGGTCTGCGCTGCGGGACGCGCGAATTGAATTATGGCCCACACCAGTGGCGCGGCGACTTCCAGTTCAACATCAGCCGCTACAGTCAACAGCAA
CTGATGGAAACCAGCCATCGCCATCTGCTGCACGCGGAAGAAGGCACATGGCTGAATATCGACGGTTTCCATATGGGGATTGGTGGCGACGACTCCTG
GAGCCCGTCAGTATCGGCGGAATTCCAGCTGAGCGCCGGTCGCTACCATTACCAGTTGGTCTGGTGTCAAAAATAATAATAACCGGGCAGGCCATGTC
TGCCCGTATTTCGCGTAAGGAAATCCATTATGTACTATTTAAAAAACACAAACTTTTGGATGTTCGGTTTATTCTTTTTCTTTTACTTTTTTATCATGGGA
GCCTACTTCCCGTTTTTCCCGATTTGGCTACATGACATCAACCATATCAGCAAAAGTGATACGGGTATTATTTTTGCCGCTATTTCTCTGTTCTCGCTATT
ATTCCAACCGCTGTTTGGTCTGCTTTCTGACAAACTCGGAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAA
ATTTAATTAAGGCCGCGGGATCGATCCCGTCGAGCAGTGTGGTTTTCAAGAGGAAGCAAAAAGCCTCTCCACCCAGGCCTGGAATGTTTCCACCCAAT
GTCGAGCAGTGTGGTTTTGCAAGAGGAAGCAAAAAGCCTCTCCACCCAGGCCTGGAATGTTTCCACCCAATGTCGAGCAAACCCCGCCCAGCGTCTTG
TCATTGGCGAATTCGAACACGCAGATGCAGTCGGGGCGGCGCGGTCCCAGGTCCACTTCGCATATTAAGGTGACGCGTGTGGCCTCGAACACCGAGCG
ACCCTGCAGCCAATATGGGATCGGCCATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCA
CAACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCGCAGGGGCGCCCGGTTCTTTTTGTCAAGACCGACCTGTCCGGTGCCCTGAAT
GAACTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTG
GCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGCA
TACGCTTGATCCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCGCATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGATG
ATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCGCCAGGCTCAAGGCGCGCATGCCCGACGGCGAGGATCTCGTCGTGACCCATGGC
GATGCCTGCTTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTCATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCG
TTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGCGAATGGGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCTCCCGATTCGCAGCGCATCGCC

TTCTATCGCCTTCTTGACGAGTTCTTCTGAGGGGATCGGCAATAAAAAGACAGAATAAAACGCACGGGTGTTGGGTCGTTTGTTCGGATCCGAATTCCT
CGAGGGCGCGCC