NIH-1149 Genotyping Strategies

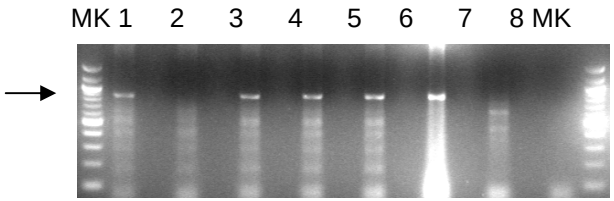
Reaction Components	Vol (ul)
10X Sigma Buffer	5
25mM MgCl2	3.5
10mM dNTPs	2
Primer 20 uM	1.5
Primer 20 uM	1.5
5 U/ul Taq polymerase	0.5
Water	31
Total mix volume	45
Tail lysate (1:20 dilution)	5
Total reaction volume	50

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

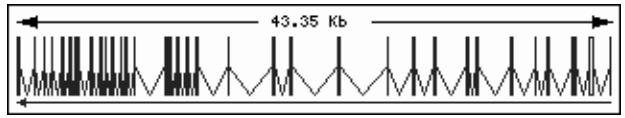
Primer Sequences (5' to 3'):	
Mutant PCR: Primer 1149-5' and Primer GT-IRES, 873 bp	
Recommended Wt PCR: Primer 1149-9 and Primer 1149-12, 421 bp	
Primer 1149-5'	ACACTCCTGTTGCGTCACACT
Primer GT-IRES	CCCTAGGAATGCTCGTCAAGA
Primer 1149-9	GGACAGGTGTATTGATTG
Primer 1149-12	GGTTTTGCAGTAGCTGTGG

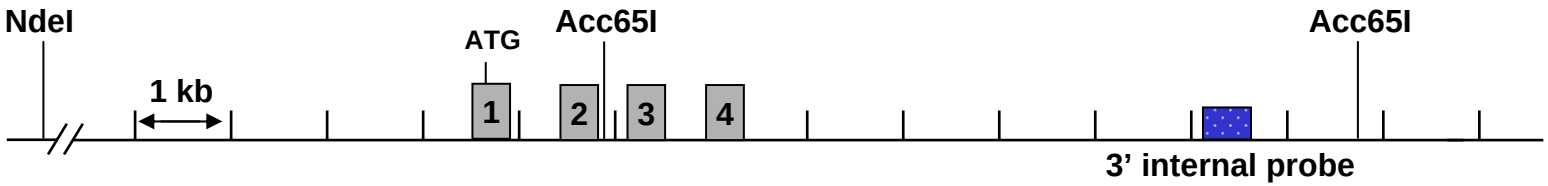
Well	Sample	Genotype
1	193	het
2	195	wt
3	198	het
4	216	het
5	223	het
6	**ES DNA**	het
7	**wt lysate**	wt
8	**water**	no amp

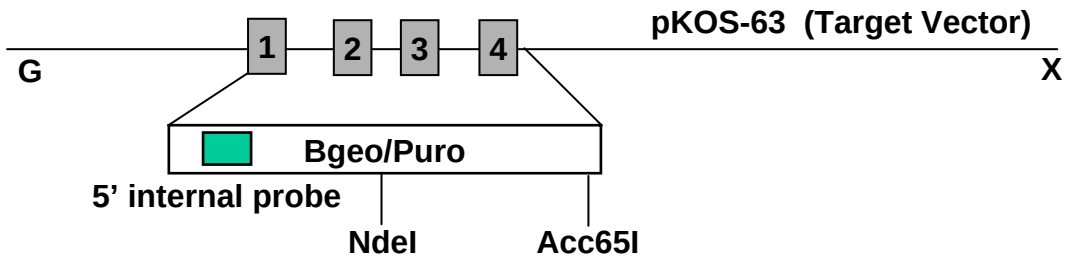
Mutant PCR



Targeting Strategy



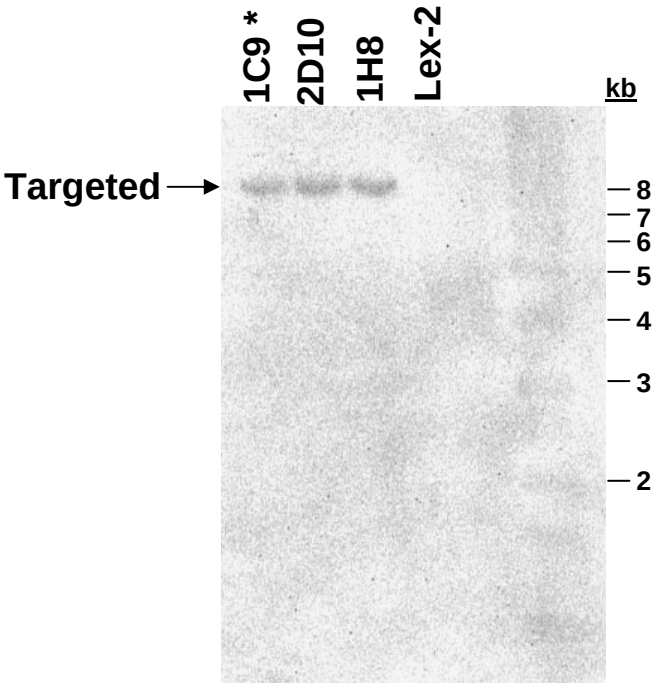




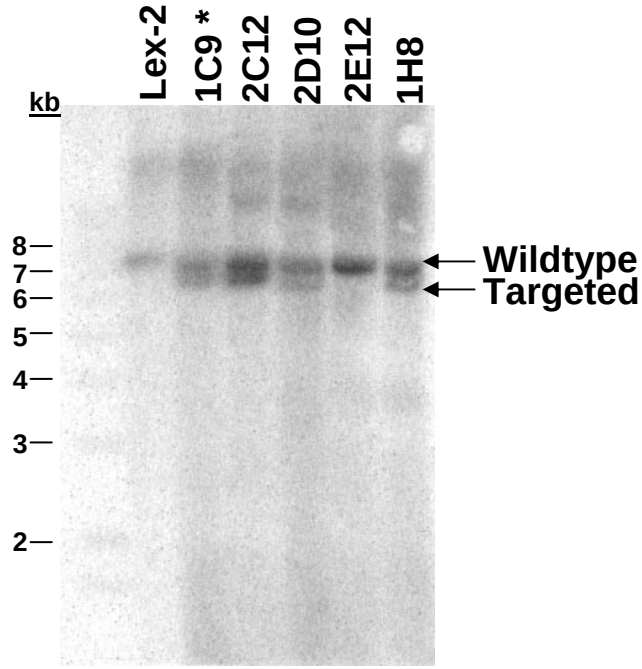
Southern Strategies

Probe	5' internal	3' internal
Enzyme	NdeI	Acc65I
Wildtype	---	7.5 kb
Targeted	7.9 kb	6.7 kb

Southern Data



5' internal probe
NdeI Digest
Targeted 7.9 kb

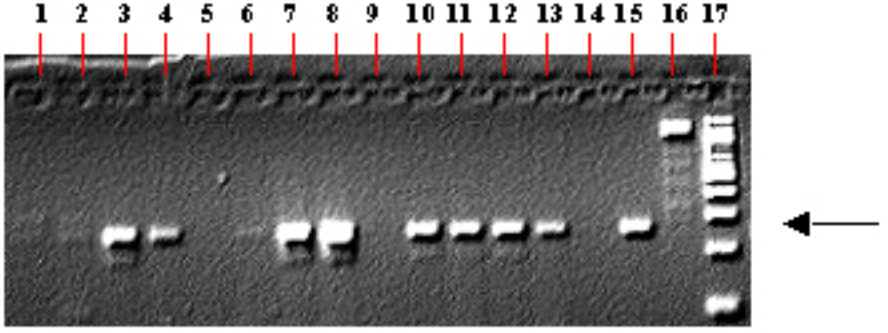


3' internal probe
Acc65I Digest
Wildtype 7.5 kb
Targeted 6.7 kb

*** Clone achieving germline transmission**

RT-PCR WT Expression Analysis

mouse random primed cDNA with Primers: 1,2



06/21/2004

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) Kidney
8) Liver
9) Skeletal Muscle
10) Bone
11) Stomach, Small Intestine & Colon
12) Heart
13) Adipose
14) (-) Control
15) (+) Control- ES cell cDNA
16) (+) Control- Genomic/Lex1 DNA
17) 100 bp ladder/marker

Lexicon Genetics Incorporated
Molecular Genetics Project Materials

Catalog Number: **_ NIH-1149** (LEXKO-032)

Reference accession(s): _ NM_007376

Standard KO or Conditional: _ Standard

Materials Submitted: ☐ Target Vector pKOS-63FTVpuro _____
☐ KOS clone(s) _ pKOS-63_____

Southern Blot Genotyping Strategies:

	5' Internal	**3' Internal**
Name of Probe:	Internal 8/9	87/87
Restriction Enzyme for Genomic Digest:	NdeI	Acc65I
Predicted Wild-type Band (kb):	--------	7.5 kb
Predicted Mutant Band (kb):	7.9 kb	6.7 kb
Probe Size:	291 bp	716 bp

Primer sequences:

Southern probes

Internal-8	5' – AGGAAGCAGTTCCTCTGGAA
Internal-9	5' – CACATGTAAAGCATGTGCACC
1149-86	5' – GACATCACTGTCCCTTACTC
1149-87	5' – CACCCTCCTCTCTTATGCTC

Genomic Sequence Deleted:

ATGAGGAGAAACCAGCTGCCCACACCAGCTTTTCTTTTACTGTTCCTGCTTCTTCCCAGAGATGCCACCACAGCTACTGCAAAACCGTAAGCTAATCTT
TTTTTTTTTTTTTTTTTGGCTTTTCAAGACAGGGTTTCTCTGTGTAGCCCTGGCTGTCCTGGAACTCACTCTGTAGACCAGGCTGGCCTCGAACTCAGAAA
TCCAACTGCCTCTGCCTCCCAAGTGCTGGGATTAAAGGCGTGAGCCACCACTGCCTGGTCTATAAGCTATTCTTCTTTCCAAAAAATTCTATTGTGTCCT
TCCTTCCTTCCCACAATACTATCTCTTTTCTGGTTTCCTAGGCAGTAATCTATTGTCAAAAAACTTGGAAGGACTTGGAAGTTGTTGGATTGAAAAGCAA
TTCAGCTTCTCCCCGTGTGTTGTGCGTTAGTTTCTAGTGCTTCCTGGATAAGATTTGTAATGCTCACATGGAACCCAAGGCCACAAGCAGTTGGGTTTTT
AAGACTCATGGTTTGGAAATTCGGAATTTCTAACTGAGACACATCATAGCTGTCGCATCAGACTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGT
GTGTGTGTGTGTGTGTGTGTATGAGGTGACACAACCTGACACAAACATTTACCCAGCCTGGAGGATAGGGAACAAACATTAGTGATTCTTCCAAAG
TCCAACTTAATAAACCAGTGTGTTCGTGGGGTGGCCTCTAGGAATGAGACAGGGGTTGCTTACAGAAACGCACATGATTCAAAGACAACTGTCACTAA
CAGGCACATTCCTGGGACTCCCTGCTTGACCTGAAGGTAGCTCAGCTGGTAGGAGAGTCTCCTCCAGGTGACTTGGCTGGTCCAGGAGTCTCTTAGAAG
CAGCTCTGCCTCCTCTCCTCTGTCTAACAGTTTTTTGCTGCCTCTGTAACCATGGAGAGACTGCATCAATCCTGTTAGCCTCCGCTCATGAAATTTACCTC
TTGTATCTCAAACCTCCCTCCTCCCTCCTCCCTCCTCCCTCCTCCGTCCTCCCTCCAGGAAGGAATGTTTCGATTTGGAGGAAATTGCTACATGGCCATA
ACTTGAATTGGTTGCCGGTGTTTCTGATATTCTGTGGGTGAACATTCCCTGAACTCCAGAGAATCACAACAATGTCTCATCTTAGGTTTTATGTTGACCT
CTGGGAAACCCAGTCTTCATGAAACCTATGATTGATGGCAATATGAAAAATACTAGAAAACAATCGCAATAATGACGTCTTCTGAATCCTTCCTGGTTG
GTCCTCTTCCCCCGCAGACAATATGTGGTGCTGGTCCCGTCAGAGGTCTATTCAGGAATCCCTGAAAAGGCCTGTGTCAGCCTCAACCATGTGAATGAG
ACTGTGATGCTCAGCTTAACTCTAGAGTATGCAATGCAGCAAACGAAGCTCCTCACAGACCAGGCTGTGGATAAGGACTCCTTCTACTGCAGCCCCTTC
ACGGTCAGTGTCCTGCCTGGGATGTGCGGTACTGGAAAAAAAAAAAAAAAGAGTAATTGAGTTGAATGTTGTATTCTCATTCATCAAAAAATATTTAA
ATTAAGGAAAAATAATGAATACAAAGTATACTTCTAATCTCCTTTATGATACTTACAAACTGGTCTAATGAAGGTTACCATAGCCATAAGAAAGACTTA
TTTTTTACTTTAAAATAAACTTTTGTGGTAAACAATATTTATACTTTTACAAATATAAATATTGAGATTCTCTTTTTTAAAGATTTATTCTTTATGTATAT
GAGTACACTGCAGCTGTCTTCAGACGCACCAGAAGAGGGCATCTGTACTCCATTACAGATGGTTGTGAGCCACCATGTGGTTGCTGGGAATTGAACTC
AGGACCTCTGAAAGAACAGTTAGTGCTCTTAGCCACTGAGCTGTCTCTCCAGCCCCTTGAGATTCTTTTTTAAGATTTATTTTCAGATTAAATTGTGTCT
CTGTGTATGTGCACATGAGTGCAGGTAACCATTGAGGCCAGAAGAGGGCACCATGGAGCTAGAACTGTGGGCAGTTGTTAGCCTCCTGATATGGATGT
GGTACCCTAATTTAGGTCCTCTCCAAAAGCAGTGTGTACCCTTAAGTGCTAAACCATCTCTCCAGCCTTTGAATACTGATCTTCTTAAAAGCCAAATTAT
TCATGTAAGATAGATGGCAGATGGTGGCCTTGGAAGGTGGAATAGGAAAATTATTTTTAATAGCAAGACTTCGCTGTGAAAATACAAGGACACCATCT
TATTCACTGCCCTGACTGCACTGAAGAATGGTCTGTTGTTCTGCAATGTTTTCTCCTAAAATCATGTTACTCGGTCACAGGAAATTGAGTCCTTGACACC
CCCTTACCAGTTCTCAAGGATTCACAAAGAAAACAGATCCCTGTAGACTCTGAGCACCCAGAAGTGAATCCGAACTTACTCTCTACGGTTTTCTGCAGA
TCTCAGGTTCACCTTTACCCTACACATTCATTACTGTGGAGATAAAAGGACCAACGCAGCGCTTCATAAAGAAGAAGTCAATACAAATAATAAAAGCT
GAGAGCCCAGTCTTTGTCCAGACAGACAAACCCATATACAAACCAGGACAGATAGGTATAAAGAATTCACTGGGCAAAAACAACACAGTGGGTGAGG
AGGGGTGGTGGTGTTCTTTACGATTAAACTGCAGTCATGGGAACTCTGGCCTTGGCTTCCCACTGTGGGGAGGTCTCACAGAAAAATCTCTGTTTCAGT
GAAATTCCGAGTTGTTTCTGTGGACATCAGTTTTCGCCCGTTGAATGAAACGGTGAGTCTCC

KOS clone sequence: *(note: KOS63 was used to generate the TV and that is the sequence included here)*

GATCTGTTCTGATTCATTGTATCCCTTTTGGTAGATTTTACACCTCTTTGGGAAATTAAGTAAATAGATGTCAACTCATTTAAGAGTGGGTCCATTCCTA
CTGGAAGAGGCCGGTGGGCAGGTGCTTGCCTGAGTGCATGTAGCTGTCGCCTCATTCCTGACAGCTAAGTGTGTCTGGGTTTGCAGTCCAGGCTCCAGT
GCCAACAGCTGCAGAGTGGGATTGGACCCTGTGACTCCCGGTCCTTCCTAAGGGAAAGGAGAGACTGGGTTTACGATTTCTTGTGTTGCTGTTCTGAGA
GCCCTGGCTTGGGGCCCATGTTCACTCAGTTGTGATATTGAGGTGGGGACCTAGCAGCTTCCAGAGCCGAAGCCAACTGAGGAATGTCTAAACAAAGT
ACTCAATTAAAAAGAAACAATTTCTATCTGGGGGAAAAAAAAGAAAGAATTTTCAAACAAAAATTTCTCCTGAACCTCCACAGGAAAGGAAAAACAT
GATCAATGAGTAGTAATATTTCAGCCCCTTTTTCTTTTAATCTGAGAGAGGACCTCACTCTGGCTTCCTGGCTGGCCTAGAACTCACTATATAGGCGAAC
CTTGTCTTGAACTGTCTGTTCTCTCCTCTGCCACACAAGTGCTGGGATTACAGGCATGTGCCACCACACCCCGCCCCACCCAGCTCTTCATTTATTTTGA
GGCAGGGTTGATAAAATTCTCAGGCTTACCTTTATCTCACTCCTGCTCCCAGGCCAACCTTAAAGTTATGTCCTCCTGCCTCAGTCCCATGAATGCCAGG
GTCACAGTCATGCACCATCATAGCCAGCAAAAGACACAAACTTTAATCTTGTGATGACTTTATATTGATGTAATCGTGGGATTTTTCTTATTTTCATGAC
TCTGTAAAGTAGATTCTAACACTCCTGTCTTGAAAAGCAAATGGGACAATGAATGAACACTGATTTAAAAAAATACTTAATTTCATTCCAGAAAAATTA
AACAGGGCTGCTCATCAAAGAGGAATTATTTTCTTAGAGAATAATCAATCTTCTTTATTTCCTTAATCTTTAGTGATTTATTAAATATGTATTGTATAAC
GTAAGTCCAAATGAACTGTCATTTACTTTATAATAAACAAAGATAAGAAGAACCATACAAAACTAAAAGCCTTCAAGGCCAGCCTGGTCTACCGAGTG
AGTTCCAGGACAGCCAAGGCTACACAGAGAAACCCTTTCTCGAAAAACAAACAAACAAACAAAACAACAACAACAACAAAACGCTAAAAGCCA
AACTCATACAGTGGAGGTTGGGCTGAGGTAGTTCAGGCTTACTGAGGACCTAAGTTTGCATCATAGGATTCACATAAAAAGACCAGGCAAGCAGGTAT
GCACCTGTGGCCCTAGTCCTAGTCCTGTGGGGCAGAGACAGGAAGAGCCCAACATCGTGCTAGCCAAACAGTCTAGCCAATTGGCGAGCTCTAGAATC
AGTGAGAGCCTCAAAAAATATGTTCAAGGCATCTACAGAGCTAATTCCAGGATATCCAGTTCTACACAAAGAAACCCTATCTCAAAAAACAAAAAAGA
GGAGGAAGAGGAGGAGGAAGAAGAGGAAGAGGAAGGAGGAGGAGGGGAGGAAAGGGAGGAGAGGGAAGGAAAATAGGGAAGTTACCTGATA
ACAATCTCTGACTGCCACACCCACATGCACCCTCAGACACACACACTCCTGTTGCGTCACACTTCTCCTAGGAAGGCCTGACACAAACATCTATTCACC
CCAGATAAGGATGTAATGACAAAACAAAGTAATGATTCTACCAAAGTCTAACTTGGCTTTACTTTTTTCAATTTCTAAAAATAAAAAATAAAATTGAAG
CACATACCTCCAAACTTACCTTTTCTAACATTCAGTGGTAAATTTCATTATGTCCTTCAGTGAATGCAATACTCCAAGTTAGGAAGAGGAAATTGATATT
GTATATTTGCATTCACTGTCCTTTTTTTAAAAAAAAATGGACAGGTGTATTGATTGACTTCACCAAACCATGTGATTTCTTTCATTTTTCTTTTAAAATGT
CTAAAGTCCTGTGTATTATTAACCGTGAGCTAGTGGCAGCCTGCAAGCACCATCTTTCAAACATTTGGGTAACTTTTAGAAGTGAATAAAAGAACATAG
ATAGTCCTAGAAAATGGTCTGGAATTGATTTTTTAATAGTTAATATGTTACAAAAATAAATGGTTGGCAGTTTACTTTCACTGCCCATCAGGTAAAATA
AAAAGCAGCGGAACTGGACAAGGATCAGAGTTCGGGGGCTGAGGGCTCAGACGTTCTTCTCTGCCCTCTCCACCATGAGGAGAAACCAGCTGCCCACA
CCAGCTTTTCTTTTACTGTTCCTGCTTCTTCCCAGAGATGCCACCACAGCTACTGCAAAACCGTAAGCTAATCTTTTTTTTTTTTTTTTTTGGCTTTTCAA
GACAGGGTTTCTCTGTGTAGCCCTGGCTGTCCTGGAACTCACTCTGTAGACCAGGCTGGCCTCGAACTCAGAAATCCAACTGCCTCTGCCTCCCAAGTG
CTGGGATTAAAGGCGTGAGCCACCACTGCCTGGTCTATAAGCTATTCTTCTTTCCAAAAAATTCTATTGTGTCCTTCCTTCCTTCCCACAATACTATCTCT
TTTCTGGTTTCCTAGGCAGTAATCTATTGTCAAAAAACTTGGAAGGACTTGGAAGTTGTTGGATTGAAAAGCAATTCAGCTTCTCCCCGTGTGTTGTGCG
TTAGTTTCTAGTGCTTCCTGGATAAGATTTGTAATGCTCACATGGAACCCAAGGCCACAAGCAGTTGGGTTTTTAAGACTCATGGTTTGGAAATTCGGA
ATTTCTAACTGAGACACATCATAGCTGTCGCATCAGACTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTATG
AGGTGACACAACCTGACACAAACATTTACCCAGCCTGGAGGATAGGGAACAAACATTAGTGATTCTTCCAAAGTCCAACTTAATAAACCAGTGTGTTC
GTGGGGTGGCCTCTAGGAATGAGACAGGGGTTGCTTACAGAAACGCACATGATTCAAAGACAACTGTCACTAACAGGCACATTCCTGGGACTCCCTGC
TTGACCTGAAGGTAGCTCAGCTGGTAGGAGAGTCTCCTCCAGGTGACTTGGCTGGTCCAGGAGTCTCTTAGAAGCAGCTCTGCCTCCTCTCCTCTGTCT
AACAGTTTTTTGCTGCCTCTGTAACCATGGAGAGACTGCATCAATCCTGTTAGCCTCCGCTCATGAAATTTACCTCTTGTATCTCAAACCTCCCTCCTCC
CTCCTCCCTCCTCCCTCCTCCGTCCTCCCTCCAGGAAGGAATGTTTCGATTTGGAGGAAATTGCTACATGGCCATAACTTGAATTGGTTGCCGGTGTTTC
TGATATTCTGTGGGTGAACATTCCCTGAACTCCAGAGAATCACAACAATGTCTCATCTTAGGTTTTATGTTGACCTCTGGGAAACCCAGTCTTCATGAA
ACCTATGATTGATGGCAATATGAAAAATACTAGAAAACAATCGCAATAATGACGTCTTCTGAATCCTTCCTGGTTGGTCCTCTTCCCCCGCAGACAATA
TGTGGTGCTGGTCCCGTCAGAGGTCTATTCAGGAATCCCTGAAAAGGCCTGTGTCAGCCTCAACCATGTGAATGAGACTGTGATGCTCAGCTTAACTCT
AGAGTATGCAATGCAGCAAACGAAGCTCCTCACAGACCAGGCTGTGGATAAGGACTCCTTCTACTGCAGCCCCTTCACGGTCAGTGTCCTGCCTGGGA
TGTGCGGTACTGGAAAAAAAAAAAAAAAGAGTAATTGAGTTGAATGTTGTATTCTCATTCATCAAAAAATATTTAAATTAAGGAAAAATAATGAATAC

AAAGTATACTTCTAATCTCCTTTATGATACTTACAAACTGGTCTAATGAAGGTTACCATAGCCATAAGAAAGACTTATTTTTTACTTTAAAATAAACTTT
TGTGGTAAACAATATTTATACTTTTACAAATATAAATATTGAGATTCTCTTTTTTAAAGATTTATTCTTTATGTATATGAGTACACTGCAGCTGTCTTCAG
ACGCACCAGAAGAGGGCATCTGTACTCCATTACAGATGGTTGTGAGCCACCATGTGGTTGCTGGGAATTGAACTCAGGACCTCTGAAAGAACAGTTAG
TGCTCTTAGCCACTGAGCTGTCTCTCCAGCCCCTTGAGATTCTTTTTTAAGATTTATTTTCAGATTAAATTGTGTCTCTGTGTATGTGCACATGAGTGCAG
GTAACCATTGAGGCCAGAAGAGGGCACCATGGAGCTAGAACTGTGGGCAGTTGTTAGCCTCCTGATATGGATGTGGTACCCTAATTTAGGTCCTCTCCA
AAAGCAGTGTGTACCCTTAAGTGCTAAACCATCTCTCCAGCCTTTGAATACTGATCTTCTTAAAAGCCAAATTATTCATGTAAGATAGATGGCAGATGG
TGGCCTTGGAAGGTGGAATAGGAAAATTATTTTTAATAGCAAGACTTCGCTGTGAAAATACAAGGACACCATCTTATTCACTGCCCTGACTGCACTGAA
GAATGGTCTGTTGTTCTGCAATGTTTTCTCCTAAAATCATGTTACTCGGTCACAGGAAATTGAGTCCTTGACACCCCCTTACCAGTTCTCAAGGATTCAC
AAAGAAAACAGATCCCTGTAGACTCTGAGCACCCAGAAGTGAATCCGAACTTACTCTCTACGGTTTTCTGCAGATCTCAGGTTCACCTTTACCCTACAC
ATTCATTACTGTGGAGATAAAAGGACCAACGCAGCGCTTCATAAAGAAGAAGTCAATACAAATAATAAAAGCTGAGAGCCCAGTCTTTGTCCAGACAG
ACAAACCCATATACAAACCAGGACAGATAGGTATAAAGAATTCACTGGGCAAAAACAACACAGTGGGTGAGGAGGGGTGGTGGTGTTCTTTACGATT
AAACTGCAGTCATGGGAACTCTGGCCTTGGCTTCCCACTGTGGGGAGGTCTCACAGAAAAATCTCTGTTTCAGTGAAATTCCGAGTTGTTTCTGTGGAC
ATCAGTTTTCGCCCGTTGAATGAAACGGTGAGTCTCCTGTTGGTGGGTGAGAAATGAGAATGAGTGTTTACAATGAGAAAAGTAAACAAATAGCTAAG
TCTGATTTTTTTAGGAAACCTGAGCTCATATAAAAGGCACCTGACTCTACCACAGAGCATTGCCTGAGAGCGTTCACGGCAGAAGCATATTTAATGGTA
GCATTGAGACAGTTCACCAACTGCATTCTTAGAGTAGATAAAGCGTTTAAAAATAAACTTGGGTCTGGTTATGCATGCTTCTAATCCCAGGAGGTGAGA
AGCTGAGAAGAAGAATCACAAGGCCCTGGCCTGCCTGGACCACATAGTAAGATGCTGTCTAAAATATAACAACAAAAAGCATATTCAGTTGAAATTGT
CCTCTTTTTTTAAAATTTTTATTAGGTATTTTCCTCATTTACATTTCCAATGCTATCCCAAAAGTCCTCCATAGCCCCCCCCCCACTCCCCTACCCACTCA
CTCCCACTTCTTGGCCCTGTCGTTCCCCTGTCCAGCCACTTCTAGAAGCCCATTCGGTTTCTCTTAGAAATCATTAGATTTCCTCAAATGTATGCTAGCCA
TAAAAGGAAAGCCATGACTTCTTAAGAGATTCATGTTCCAAACTAGACCTGTAAAAAGGTAATGGACCAAACAGAGGACGTCTCCTACTTTAGACAAG
AAACACAATGACTTTGTCTCTCTGCCATGTAGGCTCTGTCACCTGATGTCATGGTTATCTCTGACGTCCATGACTATGAGTGCAGCACCATTGGACCTGA
TGGGGGGAGGGGGACACGAAGGCGGGTGGGTAGAGGAGGGAGTGTGGGAAGAGTGAATATGGTCCAAACTCATTTTACAAAATTCTCAAAGAACTAA
TTAAAAAGGACATCTGGTACAGAACAAGGAAATAAGTCTTTGTATGAAAAAGGAAGCAATAAGCTCGGTAAAGTTTAAAGACTGTAATTACAAAGAT
GCTCTCTTTTTAAACTTCACCGCACACTCATGCTGCTTTTTTTTTCTTCTCAGTTCCCTGTCGTTTATATTGAGGTAAGTACCAAACTGTACATCTCGGTC
AGAATGACGATGCCCTACAAGTTCCGCAAGATCTTTGTGTCTCCTTTCAGACTCCAGTTCCTCTCCACTAAGCAAATCCTATCTTCTCTGCTCCCTCAAG
TTCTTTGGGAAAGGAAAGGAAAAGGACCGCAGTACTTTATGATGGTGTGATTTTGTGGCTTTCCAAATATCTCTGGCTAGAACGGACATATATATCAGA
AGGAAGTCCACTGTGACACACATATGCAGGGTCTCCCTTCTAAGTACTAACCCGGTTGGCTTCTAAGATCAGACAAGACTGTAGCATCTGCGGTGGCAC
AGCTGCAGGGGTCAAAGGATCCCATCGCTGGTGACATCACTGTCCCTTACTCAGGTCTACAGTGTGTTTGTTTCTTTGTTTCTTTGTTTAGTTGGTTAAC
GACATTAGCACTAATTCACCAACTCCCTATTAACTCCCAGTGTTATTATTACCTGATAAGCTGACCTCTAATCAAATTATTTACACAATTGTCTCAATTG
TTTACACAACTCATAATATCTTTCCATAGACTCCCAAGAGGAACCGAATTTTTCAATGGCAAAATATCCATCTGGCAGGAGGACTCCACCAGCTCTCTT
TCCCACTGTCTGTTGAGCCAGCTCTGGGTATCTACAAGGTTGTAGTGCAGAAGGACTCAGGGAAGAAAATAGAACACTCCTTTGAGGTGAAGGAATAC
GGTATGAATTGGACAAGTGTAAGGCATATATGATGGTGGAATCCTCAAGGTTACTTCTCCGTATTTGTCTGTGGTATACGCGTGTGTGTGCATGTTGGG
GGTGTGGTATGTTCTCACTCCGTGTGTGCATATGTAGATGGGAGAAGTTGACATCAGGAAACCAATCATAAAACATGATTCTCAAAGCTCCCCAGAGCT
TCTATGGCAGTCACATAATACGATAAATACCTTTCACACCTACGGAGAATTTGGAAAAGGAAGGATTCAGCGGGAGGAAGTGGCAAATCACAGAACA
TGACTAGCAAGTTCTGAAGTGGGCACGGGCTACTCAGAGCATAAGAGAGGAGGGTGTGCAACTTCAGCCAGAGTGGAAGATTCCTGGGAGTCAGTGA
ATGAGTCTGCAGCCCTTCAGTTCAGTGGACCTATAACCACGTGTCTGATAATGGGGCTGAGGATGTCAGTCAGTTGGAAGAGCTAGCATTTGGTTAAAA
TGAGAGATGGGGGAGGGAGAGGGAGAAAAACATCAAACTTGAACCCACTGGCCCTACTGTTCCATCAACTAACTGTAGTGGTCTTCAGACTCAGTGAT
GTTTCACCGGCCTTTGAAATCTCGCACAAAATCAGAGGAGTCTGGGAAATGTAGTCTGGTTTCACCAAGTGGCATGTACAAACAGCTCACAGTAATAA
ATGACAATATCTCTTTCCTAGTATTGACACTAGAGCTCCTTCTGTGTGATTCTATGTCATAGATTCCTCTAATAAAGTCCACTGGTAATATTTACGAGGT
CTCCTACCTACTCTCACCTCCTTTTTTCCTCTATTCCAAACAGTTTTACCCAAATTTGAGGTGATAATAAAAATGCAGAAGACTATGGCTTTCCTGGAAG
AGGAACTTCCTATAACTGCTTGTGGCGTGTGAGTTACTTCTTTTTTCATCTTATTTTTGGTTTTCATATCCTATTTGAGATGGTATTTTAAATAATATTTAG
TAAGGCATTCCATATCAGCACTTACAGGCGCTGGACAGGGAGTACAAGCAGAAATCTATGTCCATGAGGCTAAAGAAACAACCTCACAACTGGCCTAA
GGGAAGATTCCTATATCGGCTCTACAGGAGGAGCTTAATGAAAATTAGAATCCCTGGTTCCACCTTCAGAAATTCTAGTTACAAATCTAAGGCAAGAG
TCACAAACCCTGTACCACAACATGGGTCACAGGGCATTCTAGAACAGGAAACTAGTCCCACCCTTGCCTGGTGCCCTTCCACCATATTCAGCAGAAAC
AGACTGAGCCACCACGATATTATTTACAAATCATATTTTTATTTTGTAGCCACTGCTTTCCCAAACAATTTCTCACTTATTATTTTAAAATACCTAGAAA
TATAGTCTTTATCTTTTTCTTTTTTAGTTAAAATAACTAAATCTGACATCAGAGACAATCATTTTTTAACTAATTCTGTGGTATATACATATGTGCTACAT
ACATGAGCAAATGCCCAAGAAGCCTAGAGGTAGTAGAGACATCAGAGACAATCATTTTTTAACTAATTCTGTGGGTATATACATATGTGCTACATACA
TGAGCAGATGCCCAAGAAGCCTAGAGGTAGTAGAGGCACTGAAGCACTAAAACTGGAATTGCAGGCAGCTGTGAGCCACCCGACACGGGTGCTGGGA
ACAAAACTCAGGTTGTTTGGAAGAGCGGGAAGCACTCTTATTTTATGTGTATGGGCGTTGTGCCCACACATGTGTCTGTGTCCCACATGCATGCCTGGT
GCCTGCAGAGACCAGAAAAGGGCATCAGAGTCCTTGGAAGTTGCAGATGATTCTGAACCATCAGTTGGGTGCTGGGAATTGAACCTGGGTCCTCTGGA
GGAGCAGCAAGTGCTCTCTTGTCTACCATCTTTCCAGTGAACAGGACAGTCTTATAGCAGAACCTCAACTAAAAGGAGAGAAAGAGCTGGGTGTGGTG
ACCTTTAATCCCAGTAATCAGGAGACAGAGGCAAGCAGATCTCTGAATGTACATCAGCCTGGTATAGATAGCAAGTTCTAAGCCAGCCCAGAATACAT
ATTGAGACCTTGTCTCCAAAGCTTAAAAAAAAAAAGAGAGAGAGAAGGAAGGAAGGAAGGGAGGGAGGGAGGGAGGGAGGGAGGAAGGAAGG
AAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGAAAAGAGAAGCAAAAAAAGAAGGAAGGAAGGAACCTTGGGAACACTAAATC
GTATCTTTCCATTTAGAGATTTTTCCAAAAGGTTCGCTGACTACTTTGTCTTAAAATTATTTCTCATTTGCACTTAGGATGAAGCCTGGTATCACGAGCCT
TACTGGAGTGCAAACACTAGAGAGATAAACAAATATGGTTGAACAGTGTGACTGGGTCCAGTCAGGGAAAGAAACCATCCGTGACACTCACATTCGAT
CATGTTACTGTTCTAATACACATATGGAAAGCCTGTTCCCGGTCTGGTGACATTGAGAGTGTGCAGAAAATATTCACGATACCGCTCCACCTGCCATAA
CCAAAACTCAATGAGTATCTGTGAAGAATTCAGCCAACAGGTATGTAGTAGACATTCCCTAGTTCTTACTCTGGCATGAGCTGCAGTGCTAAGTGCAAT
GCAATAGGACAAACACTTGAAGTGTCATCAAGCAAGATGTAGGAGTAGGGAGAAGAAATATGAGAATGGTTGGTGGTATTAGGGGCCCAGATGAAGG
CAGAGATTATGAATCTATAACTGCGCCCATTTGGCAAATTTTGTAATTTTCTTGCCAACAGCCATCAACATGCCACGTTCTAAAAGAATAAAGTCGTGT
TCAAAGCTGGCTTTTGTCACAGTAGAATGAGGTCGAAGCTGATGTGGGCTGAATGAGAACTAGGCTCAAAAAGAACTAGTGGCCCTGGAGAGACGTCT
CAGTAGCTAAGAACACTTGTTGCTCCTGCTGAGGGTCTAGGTTCAATTCCCAACACACACACACACACACACACACACACACACACACACACACAC
ACACACACACACGGCAACACACAACCTTCTGTAACTCCAGTCCCAGGAGTGTTCTAGCCTCTGTAGGGTTTGCATGCACGTGCTACATAGACACAC
ACGCAGGCAAAACACCTACAAACATAAAATAAAATTAAAGAATCTTAAAGTTGTATGCATGCGTTCTGATTTCAGAGGAAGGATAGGTAAAAAAATTC
AGGGATGGCTATTAGAGCCAGCTCTGTATAGAAACTGCAGGAAAGTTACCAAAGCTTCAGGGAGCCAATCAGTCAGAGGCCAAAATGGAAGGTGTGT
GGATAAATGGGACAAACACTGATAAATAACATCAGTTCACACTCTGGCTGTTTAGAAAATGACTCATGGTGTCTATAAATCCATTAATTATGATGGGGT
TTGGAGGAAGTGTATGTGTGTGTGTGTGTGTGTGCATGCGCGCACACTGAGACAAATTTTATAGCCCAGGCTAGCCTTAAATGAACTATGTTTTCCAAA
CTGACCTCAAATAATTCTCATGCTCCAACCTCCCAAATGCTGGGATTACAAATGTTTGTCAACATGAACTATGATATGATTAAATTTGTCATGCAATGA
ATATTTGCATGTAAGTATGTACAAATAGGAAACAAATCTTTATTAAAACATAGGTCACCACATTTAATTCAGAAACCTGATTGCTATAAGGATTTGCCT
AGCCAATGGCTATGCATATACCAATGTCTTAGACACCAAGAGAATACAGAAAGTAACAAATGCTGGCAAGGACACAGAGAAAACGGAACCCAAGTGC
AGTGGGGATGTAAGCTACTGCAGCCACTAAGAAACTCAGAAGGGAAGTTCAAAAAAAATAAAAATAGATCTATCATATGAAATACCTGGGTATTTGCC
CAAAGGATTCAAACACCACAGAGTCAACATGCCACGCCGGCACATCCACAGTTATTACAGCAGTGCTCTCAACAGCTATGTTATAAACCAGCTGAAGT
GTCTGTCAGTAGAGGAGTGGATAAAGAAACTATGGTGTGATC

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

BGeo/Puro

GGCGCGCCGGATCCGAATTCCTCGAGGCTAGAACTAGCGATAAGCTTCGAGCGGGATCAATTCCGCCCCCCCCCCTAACGTTACTGGCCGAAGCCGCTT
GGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATTTTCCACCATATTGCCGTCTTTTGGCAATGTGAGGGCCCGGAAACCTGGCCCTGTCTTCTTGACG
AGCATTCCTAGGGGTCTTTCCCCTCTCGCCAAAGGAATGCAAGGTCTGTTGAATGTCGTGAAGGAAGCAGTTCCTCTGGAAGCTTCTTGAAGACAAACA
ACGTCTGTAGCGACCCTTTGCAGGCAGCGGAACCCCCCACCTGGCGACAGGTGCCTCTGCGGCCAAAAGCCACGTGTATAAGATACACCTGCAAAGGC
GGCACAACCCCAGTGCCACGTTGTGAGTTGGATAGTTGTGGAAAGAGTCAAATGGCTCTCCTCAAGCGTATTCAACAAGGGGCTGAAGGATGCCCAGA
AGGTACCCCATTGTATGGGATCTGATCTGGGGCCTCGGTGCACATGCTTTACATGTGTTTAGTCGAGGTTAAAAAAACGTCTAGGCCCCCCGAACCACG
GGGACGTGGTTTTCCTTTGAAAAACACGATAATACCATGGGGGATCCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAAT
CGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATG
GCGCTTTGCCTGGTTTCCGGCACCAGAAGCGGTGCCGGAAAGCTGGCTGGAGTGCGATCTTCCTGAGGCCGATACTGTCGTCGTCCCCTCAAACTGGCA
GATGCACGGTTACGATGCGCCCATCTACACCAACGTGACCTATCCCATTACGGTCAATCCGCCGTTTGTTCCCACGGAGAATCCGACGGGTTGTTACTC
GCTCACATTTAATGTTGATGAAAGCTGGCTACAGGAAGGCCAGACGCGAATTATTTTTGATGGCGTTAACTCGGCGTTTCATCTGTGGTGCAACGGGCG
CTGGGTCGGTTACGGCCAGGACAGTCGTTTGCCGTCTGAATTTGACCTGAGCGCATTTTTACGCGCCGGAGAAAACCGCCTCGCGGTGATGGTGCTGCG
CTGGAGTGACGGCAGTTATCTGGAAGATCAGGATATGTGGCGGATGAGCGGCATTTTCCGTGACGTCTCGTTGCTGCATAAACCGACTACACAAATCA
GCGATTTCCATGTTGCCACTCGCTTTAATGATGATTTCAGCCGCGCTGTACTGGAGGCTGAAGTTCAGATGTGCGGCGAGTTGCGTGACTACCTACGGG
TAACAGTTTCTTTATGGCAGGGTGAAACGCAGGTCGCCAGCGGCACCGCGCCTTTCGGCGGTGAAATTATCGATGAGCGTGGTGGTTATGCCGATCGC
GTCACACTACGTCTGAACGTCGAAAACCCGAAACTGTGGAGCGCCGAAATCCCGAATCTCTATCGTGCGGTGGTTGAACTGCACACCGCCGACGGCAC
GCTGATTGAAGCAGAAGCCTGCGATGTCGGTTTCCGCGAGGTGCGGATTGAAAATGGTCTGCTGCTGCTGAACGGCAAGCCGTTGCTGATTCGAGGCG
TTAACCGTCACGAGCATCATCCTCTGCATGGTCAGGTCATGGATGAGCAGACGATGGTGCAGGATATCCTGCTGATGAAGCAGAACAACTTTAACGCC
GTGCGCTGTTCGCATTATCCGAACCATCCGCTGTGGTACACGCTGTGCGACCGCTACGGCCTGTATGTGGTGGATGAAGCCAATATTGAAACCCACGGC
ATGGTGCCAATGAATCGTCTGACCGATGATCCGCGCTGGCTACCGGCGATGAGCGAACGCGTAACGCGAATGGTGCAGCGCGATCGTAATCACCCGAG
TGTGATCATCTGGTCGCTGGGGAATGAATCAGGCCACGGCGCTAATCACGACGCGCTGTATCGCTGGATCAAATCTGTCGATCCTTCCCGCCCGGTGCA
GTATGAAGGCGGCGGAGCCGACACCACGGCCACCGATATTATTTGCCCGATGTACGCGCGCGTGGATGAAGACCAGCCCTTCCCGGCTGTGCCGAAAT
GGTCCATCAAAAAATGGCTTTCGCTACCTGGAGAGACGCGCCCGCTGATCCTTTGCGAATACGCCCACGCGATGGGTAACAGTCTTGGCGGTTTCGCTA
AATACTGGCAGGCGTTTCGTCAGTATCCCCGTTTACAGGGCGGCTTCGTCTGGGACTGGGTGGATCAGTCGCTGATTAAATATGATGAAAACGGCAACC
CGTGGTCGGCTTACGGCGGTGATTTTGGCGATACGCCGAACGATCGCCAGTTCTGTATGAACGGTCTGGTCTTTGCCGACCGCACGCCGCATCCAGCGC
TGACGGAAGCAAAACACCAGCAGCAGTTTTTCCAGTTCCGTTTATCCGGGCAAACCATCGAAGTGACCAGCGAATACCTGTTCCGTCATAGCGATAAC
GAGCTCCTGCACTGGATGGTGGCGCTGGATGGTAAGCCGCTGGCAAGCGGTGAAGTGCCTCTGGATGTCGCTCCACAAGGTAAACAGTTGATTGAACT
GCCTGAACTACCGCAGCCGGAGAGCGCCGGGCAACTCTGGCTCACAGTACGCGTAGTGCAACCGAACGCGACCGCATGGTCAGAAGCCGGGCACATC
AGCGCCTGGCAGCAGTGGCGTCTGGCGGAAAACCTCAGTGTGACGCTCCCCGCCGCGTCCCACGCCATCCCGCATCTGACCACCAGCGAAATGGATTT
TTGCATCGAGCTGGGTAATAAGCGTTGGCAATTTAACCGCCAGTCAGGCTTTCTTTCACAGATGTGGATTGGCGATAAAAAACAACTGCTGACGCCGCT
GCGCGATCAGTTCACCCGTGCACCGCTGGATAACGACATTGGCGTAAGTGAAGCGACCCGCATTGACCCTAACGCCTGGGTCGAACGCTGGAAGGCGG
CGGGCCATTACCAGGCCGAAGCAGCGTTGTTGCAGTGCACGGCAGATACACTTGCTGATGCGGTGCTGATTACGACCGCTCACGCGTGGCAGCATCAG
GGGAAAACCTTATTTATCAGCCGGAAAACCTACCGGATTGATGGTAGTGGTCAAATGGCGATTACCGTTGATGTTGAAGTGGCGAGCGATACACCGCA
TCCGGCGCGGATTGGCCTGAACTGCCAGCTGGCGCAGGTAGCAGAGCGGGTAAACTGGCTCGGATTAGGGCCGCAAGAAAACTATCCCGACCGCCTTA
CTGCCGCCTGTTTTGACCGCTGGGATCTGCCATTGTCAGACATGTATACCCCGTACGTCTTCCCGAGCGAAAACGGTCTGCGCTGCGGGACGCGCGAAT
TGAATTATGGCCCACACCAGTGGCGCGGCGACTTCCAGTTCAACATCAGCCGCTACAGTCAACAGCAACTGATGGAAACCAGCCATCGCCATCTGCTG
CACGCGGAAGAAGGCACATGGCTGAATATCGACGGTTTCCATATGGGGATTGGTGGCGACGACTCCTGGAGCCCGTCAGTATCGGCGGAATTCCAGCT
GAGCGCCGGTCGCTACCATTACCAGTTGGTCTGGTGTCAGGGGATCCCCCGGGCTGCAGCCAATATGGGATCGGCCATTGAACAAGATGGATTGCACG
CAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCGC
AGGGGCGCCCGGTTCTTTTTGTCAAGACCGACCTGTCCGGTGCCCTGAATGAACTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGC
GTTCCTTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTCCTGTCATCTCACCTT
GCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCG
CATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCGCCA
GGCTCAAGGCGCGCATGCCCGACGGCGAGGATCTCGTCGTGACCCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGA
TTCATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGCGAATGGGCTGA
CCGCTTCCTCGTGCTTTACGGTATCGCCGCTCCCGATTCGCAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAGGGGATCAATTCTCTAGA
GCTCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTACCTTCCTTGACCCTGGAAGGTGCCACTCCCACTG
TCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGAT
TGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGCTTCTGAGGCGGAAAGAACCAGCTGGGGCTCGATCCTCTAGAGTCGAGTACC
GGGTAGGGGAGGCGCTTTTCCCAAGGCAGTCTGGAGCATGCGCTTTAGCAGCCCCGCTGGGCACTTGGCGCTACACAAGTGGCCTCTGGCCTCGCACA
CATTCCACATCCACCGGTAGGCGCCAACCGGCTCCGTTCTTTGGTGGCCCCTTCGCGCCACCTTCTACTCCTCCCCTAGTCAGGAAGTTCCCCCCCGCCC
CGCAGCTCGCGTCGTGCAGGACGTGACAAATGGAAGTAGCACGTCTCACTAGTCTCGTGCAGATGGACAGCACCGCTGAGCAATGGAAGCGGGTAGG
CCTTTGGGGCAGCGGCCAATAGCAGCTTTGCTCCTTCGCTTTCTGGGCTCAGAGGCTGGGAAGGGGTGGGTCCGGGGGCGGGCTCAGGGGCGGGCTCA
GGGGCGGGGCGGGCGCCCGAAGGTCCTCCGGAGGCCCGGCATTCTGCACGCTTCAAAAGCGCACGTCTGCCGCGCTGTTCTCCTCTTCCTCATCTCCGG
GCCTTTCGACCTGCACTGCGCGCCAGCTTACCATGACCGAGTACAAGCCCACGGTGCGCCTCGCCACCCGCGACGACGTCCCCAGGGCCGTACGCACC
CTCGCCGCCGCGTTCGCCGACTACCCCGCCACGCGCCACACCGTCGATCCAGACCGCCACATCGAGCGGGTCACCGAGCTGCAAGAACTCTTCCTCAC
GCGCGTCGGGCTCGACATCGGCAAGGTGTGGGTCGCGGACGACGGCGCAGCAGTGGCGGTCTGGACCACGCCGGAGAGCGTCGAAGCGGGGGCGGTG
TTCGCCGAGATCGGCCCGCGCATGGCCGAGTTGAGCGGTTCCCGGCTGGCCGCGCAGCAACAGATGGAAGGCCTCCTGGCGCCGCACCGGCCCAAGG
AGCCCGCGTGGTTCCTGGCCACCGTCGGTGTCTCGCCCGACCACCAGGGCAAGGGTCTGGGCAGCGCCGTCGTGCTCCCCGGAGTGGAGGCGGCCGAG
CGCGCCGGGGTGCCCGCCTTCCTGGAGACCTCCGCGCCCCGCAACCTCCCCTTCTACGAGCGGCTCGGCTTCACCGTCACCGCCGACGTCGAGGTGCCC
GAAGGACCGCGCACCTGGTGCATGACCCGCAAGCCCGGTGCCTGACGCCCGCCCCACGACCCGCAGCGCCCGACCGAAAGGAGCGCACGACCCCATG
CATCGATGATCTAGAGCTCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAG
GTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACA
GCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGCTTCTGAGGCGGAAAGAACCAGCTGATTACCCTGTTA
TCCCTACTCGACCTCGAGGGCGCGCC