GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

mmrrc@ucdavis.edu

530-754-MMRRC

NAME OF PCR: **B6;129S5-*Bpifb1*^{tm1Lex}/Mmucd** **MMRRC #** **032698-UCD**

Protocol:

Reagent/Constituent	Volume (µL)
Water	10.275
10x Buffer	2.5
$MgCl_2$ (stock concentration is 25mM)	1.7
Betaine (stock concentration is 5M) *Optional*	6.5
dNTPs (stock concentration is 10mM)	0.5
DMSO *Optional*	0.325
Primer 1 (stock concentration is 20µM)	0.5
Primer 2 (stock concentration is 20µM)	0.5
Primer 3 (stock concentration is 20µM)	0.5
Primer 4 (stock concentration is 20µM)	0.5
Taq Polymerase 5Units/µL	0.2
DNA (50-200ng/ µL) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
TOTAL VOLUME OF REACTION:	**25.000 µL**

Comments on protocol:

- Protocol may work with other DNA extraction methods.
- Use Touch-Down cycling protocol-first 10 cycles anneal at 65°C decreasing in temperature by 1.0°C; next 30 cycles anneal at 55°C.
- Betaine and DMSO have been standardized due to high GC content. Protocol may be tested without. Also, may adjust $MgCl_2$ to increase reaction or decrease non-specific amplifications.

Strategy:

Steps		Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting	HOT START? ☐	94	5:00	**1**
2. Denaturation		94	0:15	
3. Annealing	steps 2-3-4 cycle in sequence	65 to 55 (↓1°C/cycle)	0:30	**40x**
4. Elongation		72	0:40	
5. Amplification		72	5:00	**1**
6. Finish		15	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1. Neo3A	GCAGCGCATCGCCTTCTATC
2. DNA114-16 (Corrected Primer)	CAACATCAGCTAGGCAGTCAACG
3. DNA114-14	GACAGACCAGACTAAGTC
4. DNA114-15	CCCTACTGTCCTGTCATC

Electrophoresis Protocol:

Agarose: 1.5% **V:** 90

Estimated Running Time: 90 **min.**

Primer Combination	Band	Genotype
1 and 2	419 bp	KO
3 and 4	272 bp	WT

The Lexicon PCR Protocol is attached below.

Please note: the primer DNA114-16, CGTTGACTGCCTAGCTGATG listed on the Lexicon Protocol is incorrect. Use the primers listed above to ensure correct results.

Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ706	**Date of Submission:**	3.1.04.
Lexicon Contract Name:	DNA114	**Mutation Type:**	☒ Standard Knock out
LexVision Name:	SEC464N1		☐ Conditional
Reference accessions:	ENSMUST00000028987, NM_153418	**Is this gene X-linked?**	No

---

Required Materials:
☐ pKOS clone DNA(s) pKOS-92
☐ Target Vector DNA pTV-92
☐ Targeted ES Cell DNA 1G11
☐ Genomic Map

Southern Blot Analysis:
External/Internal Probe Strategies

	5' External	**3' External**
Name of Probe:	**8+9**	**12+13**
Restriction Enzyme for Genomic Digest:	**BglII**	**ApaI**
Predicted Wild-type Band (kb):	**11.9 kb**	**7.8 kb**
Predicted Mutant Band (kb):	**17.2 kb**	**11.1 kb**
Probe Size:	**156 bp**	**129 bp**

PCR Strategies:
For standard knockouts, give wildtype and mutant-specific strategies
For conditionals, give 5' loxP and cre-excision strategies

Wild type-specific (absent in targeted allele)		**Mutation-specific product (absent in wt)**	
5' Primer Name:	14	5' Primer Name:	Neo3A
3' Primer Name:	15	3' Primer Name:	~~16*~~ —DNA114-16
Predicted Wild-type Band (bp):	272 bp	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	~~459 bp *~~ 419 bp

5' loxP strategy		**Distinguish Cre-excised and wt**	
5' Primer Name:		5' Primer Name:	
3' Primer Name:		3' Primer Name:	
Predicted Wild-type Band (bp):		Predicted Wild-type Band (bp):	
Predicted mutant band (bp)		Predicted mutant band (bp)	

Primer sequences: *Please note: the primer sequence forDNA114-16 is incorrect. It should be 5' CAACATCAGCTAGGCAGTCAACG. The MMRRC at UC Davis recommends using the protocol above to ensure correct results.

Southern probes
DNA114-8 5' – GAGACACCAGACATCAACC
DNA114-9 5' – CCTCTGCTTCCTTCTGAG
DNA114-12 5' – GCTGTGTCGATGTTAGGC
DNA114-13 5' – GTCAGACTCTAGGACAGAG

PCR Genotyping
DNA114-14 5' – GACAGACCAGACTAAGTC
DNA114-15 5' – CCCTACTGTCCTGTCATC
~~DNA114-16 5' – CGTTGACTGCCTAGCTGATG*~~ Corrected primer DNA114-16 5'- CAACATCAGCTAGGCAGTCAACG
Neo3A 5' – GCAGCGCATCGCCTTCTATC

Genomic Sequence Deleted:

GGCACTGAAGGACCATGATGCCACTGCCATCCTCCAGGAGTTGCCACTGCTCAGAGCCATGCAAGATAAGTCTGGCAG
TATCCCCATACTGGACAGCTTCGTGCACACCGTTCTGAGATACATCATATGGTGAGTGGGCCCGACAGCATTGGGAGCC
ATCTCTTCCCCACACCTCTGCCTACACATTGTGCAGCTGTCAATCAACAGTTATGAAGACAGACCAGACTAAGTCTCTC
TTTTGATAAGCCCCCAGAATGGCTAGTGGTGTCCATAAGGCAGTGTCACTGGTACCTCTAAGAGGGAAGCCACTTAGGA
AGGTTCCCCAGAGTGCTGGAACCTGGAGGTGCTTTTTAATGGAGGTCATGGTTAAGCCAGGGACACCAAGAAACTGAT
GTTTCAGGCAAAGGGGCCAGTGGGGCAAAACCATGGAGGGGCAAGAATCACACAATTCGAACCCCAACAGCAAAGGA
TGACAGGACAGTAGGGTCAGGTCATGGAGGAGCTCTGTCAGCCCAACAGGCTGAGGCTAGAAGCCGAGGAACCTGAA
GAGAAGCAAGCGAGGGTGAAGGGGAGAGACAGAGGTAGGGCCATGATGGAAAGGATTAGTAAACTAGGCTTGGGATC
CAGGCTCTGCTCAGGTGTGAGGAAGTCAGGGGAAAGGATGGGAGCAGGCTTGGGGCTGACTCTCGTCCGAGTGTGAAC
ATGATTCTCATCCCCCTGCACCCTGTCCAGGATGAAGGTCACCTCTGCTAACATCCTCCAGCTGGATGTGCAGCCTTCA
ACTTATGACCAGGAGCTGGTGGTCAGAATCCCCCTGGACATGGTGGCTGGACTAAACACGTGAGTGTCCCCTGAGTCA
GACACAGCTAACCTAACAGTTTCAGCTACTCGCTCTCCATGCAGATGTCTAGAAACAAATCCCAGCATTAGATGAACAA
GTGCAAAGGCCCTGAGGCAGAAATGACAACAAGAAGGGAAGGGGTGGGGTTAGAGATGAGGTCAGCAAGCCGCTGAG
GACTGATGGTGCCGGACCTTATGTGACAAGATGAGAACTAGGCTTTAATGGAATATGAGGGCCAAAAGCAACAGAAGG
TCTGAGCTTAGGGGAAAACAGTGTGGTGCAGAGTGGAAGTGGGACCACCACATGGAAGGCCCTGCTCTAGGGGAGAC
AGAAGTGGCTGAGGTAGCCAGGCAGTGGTGGCACACACCTTT:AATCC:CAGCACTTGGGAAGCAGAGGCAGGCGGATT
TCTGAGTTTGAGGCCAGCCTGGTCTACAAAGAGAGTTCCAGGACAGCCAGGGCTATACAGAGAAACCCTGTCTCGGGG
AAAGAAATAAAAAGTGCCTAAGGCCCATGTCTGGGATGATGGAGCAGAGGAGGAAACAGCCTCTAGCTATGAGTTGG
AAGCAGGCAGAAACAACAGGGATTCCTGTGAGTTGGATATGGAGAGAGATCAAGGATGACTTCAGGGTCTGCTCCCAC
AACAATCCGGGAGGAACAGCCAGCAGCCAAGACGACAAGGTTTGAAAGAGGCAGGCCAGGAGACAATGTGGTGTGAC
TTGGAATCCCCTGGCATCCATAAGTGGTGAGATCCGGAGAAAACTCTGGGACTCCAGATCTGGGAGAGATAATGGGAA
CCAGCAAAAGAGATAGTGAAGGACAGATAGACTGCTGTGGCACACAGACTTCAACAGCACCCTTTCTCACTGCTGTTG
TCCCCTCAGACCACTGATCAAGACCATAGTGGAGTTCCAAATGAGCACCGAGGTCCAAGCCCTCATCCGGGTGGAGAG
GAGCAAGAGCGGCCCCGCCCACCTGAATCTCAGCGACTGCTCCAGCAATGAGAGCACCCTGCGCCTCAGCCTGCTTCA
CAAGTGAGTGTTGGGCTCCTGGTCGGAGGG

Genomic Locus: (*The deleted sequence represents nt 6379-8280 in the sequence below. KOS92 used to generate the FTV represents nt 115-9704 in the sequence below.)*

AAACCACACTTTCCTCCATGACCAGCCAGAGACTGTATGACCTCAGGGACACTGCCCCCTCTCTCATCACCATACAGAT
GGGGACACGACTTAACAATGGATCTCCTAGCCCTAATCCATAGTTTACCACTTCCAGATAGAAGCCAAGACAAGCGCC
CAAAGGTCACATCAATATCCCTACTGACCCTAACATTAACCAGGTGTCACCAGGGCAGAGTGGTCAGGAGTGTGTGGC
CTGCACTAGGTGAATGGGCTCAAGGCCTCTCCTTGACACCCCTGGTCCTGTGACCTTGAACAAGTCACTATCTGCTGAG
TCTCCAGTGCTCCTGGAAAAAGCAGAATTGTTAATTCCCATCTGGTGCTGTCTGGCTAAGTGTGTCCAGGCAAGGATGA
CAAGGGTCCCTCTGGCCAGTTCACTCTTGTCCCTGGGCCCATGGTTCATCGCTCATTATTCTTTACCTCCACAGACATCT
ACTGCCTTCCTGGTGGTATAAATGAAGCCCATAGCTCAGCCTAGTAGACAGCACTACCATGAACCTCCATTGTACCGAG
GAGGTTATAGAGGCCCTGAGAGGTGAAGCCCTTTGCCCAGGGAAGCACAGCAAAGAAGTATCAGAGCGGAGGAGGGG
GATTCACACCTATTTTCTTAAAGGAATCAGTGAGCAGAGATGTTGTGTCCTTGCTGCATAGAAGTGGATGTGATGGGCA
AGGCCATACCTCCCTCCCTTCCATGTGGTTGCCCTGACTAAACTGTCTAATCACTTCTCTATAAACCTCATCCTGTTGTA
AGCTGTAGCAATGGCAACAGCCCCAAACGCACTGCTACTGAGCATCCCTCCGAGTAGGCCACATCCCCAGCTATATGC
CCTGTAAGACAGAGGAGCCTGCAAATGCCCATTTCACATATGGAAACTAAGAGGCTCAGAGTCCCAAGTCACTTCGAG
TGAATAAGGTGAAGTCAGGGCTTGAACCTGCTTTGTCCCCTAACACACATCCTGCACCTACCATGCTGGTGAGATGTCC
CCAGATGGCTTTTGAAGCCAGATTATTTCTGCTTGGACTCGTGGCTCTTCCGTTTCCTGACTGTGGAGGGCAGTTCCTCC
TCACTAATAGCAGGATGACTGGCTTCCCACGCACGAGGATGATACCCACCAACCTAACTGATAGAAAACACAAGGTGA
CATCTGCCATGCTGGAGATGTTCGTCCATCGTGACTACTGCTGTGCTCCTCATAGTCCTGTAAGAGGCAGGGGGTCCTT
GTGCTCACAGATAAGCTAGGGGAACCAGGACTGTTAAACTCACAGTTGTTCCTTCAGGGAAAGAAATGCATAGCCTGT
AATCCACCCAAAAGCCTGGGAGAGGACATGTTTACTAGCATGGCGACCTTTGCTCTGTCTGTATGGCTAGAGACTTTTC
CAGTGCCAGATCCTCCCCAAGACCCTTACTGTGAATTTGTTTTCCTCAGTTAATCCATAGAAGCTATCTTTATGGACTTT
ATAAGAGTTCCAATACAGTCATGACTCATCTTCATTTAGCTTACTTTGTTCCTCGGGGAGACTTACATATGCTTTGTTGT
GGATGTGAAATTGATTTCATTATCGCCAAATAAGTTTTCACCAAATAGTGCTGTGCTTAAATATGCCTAAAATAGCCAA
CTCAGGTCAGATTTCAGAAATTTGAGCCAACGCCGGCTAGAGTCACATTGAATAGAACTGCCTTCTTGTCCCCCATGGA
TCCTCTCTCTTCTGGCTGTAGAGGTTGCAGAGACCACTACGTGGTCCTGTGATGGTGACTTTCCAGGAATCAGAGAGCC
CTTGTAAGGTTTGCTGCATGCCTGCCATATGCCTTAGTGCTCCCTCTGTCCCTCCAGCCAGCTTGGATCCTCCTTTCTCCA
AACCTCAGGAAGCCAGAGTTCTGAGGCTGCTTCCAGGAAGGGCTTTGGTTCACCAAGAGCTGCAAATATTTACCTTGGA
GACCTCAGCCCAGCCTGAGGTCTTGGCCCACACACTCAGGGTGGCTGCCCAAGTCAGGATGCAAGATATAAGGCTGGG
TCTCTAAACCCACCACCCAGGAGAGGAGCAGACAGACCCAGGACTCTGGCACATTCAGGTAAACCGTGAGGAGGGTCT
AGAAGCCTGGACAGTCGGGAAGGTTCCTCACAGGGAGGAGTCACAGAGGTTAGCCCAATTGCTGGGCGAGAATTGAGC

CTAATAATGCCTCTGTCAGCTATGGTTTCTGGGAGTCAAAGAGAGAAGCAGGGAGGTGCTGGCATTCTCATCCAAAAG
CCATAAACAAAGATGGAAGCATGAGACAGGGTGGTCTTTTCTAGTCCTCAGTCTAGGGACTTCAAACCACTCTGCACAC
AGTGGCTGTGCAATGGCTGTGTGTGCTAAGCTGTTGCTGGGGACAAAGAGAAACATCTGTCACAACAACCCTCAAAGA
ACTCCTAGGTGACCATTGTGGGCTTACTGTGTTTAAGGAAGGCCCAGTAATAGAGAAGGAGTCAGAGACGCCTTTCTGG
GAGAGATGGTCTATAAGATGGAGAGCTGAGTGGATGCTGGAGAGGAGCAAAGGCATCCATGAAAAAGGGAAAGGGGT
TGAGGACCACATAGTGGGTCCATGATGAGGACAGAGATTGTGGATCACATAGTGGGTCTAAAAAGAAGGCAGACATTG
AGGACCATGTAATGAGTCCATAATGAAGGCAGGGACTGAGGACCACATAGTAGGTCCATGAAGAGGGCAGGAGATGA
GGAATAAATGGTTGATGGGCACAGGCTGGGACTTGGGGGAGACAGTATCTCCCAGAAGAAGCAATGCTACAGGTTCTG
GGTTTAGAGCCCCTCCGATCCAGAGGCTGAGCAGGGGGCAGGAGAAGAGGACAGACAGGATCCAAAGTGTTTGGAAG
ACAAAGACACCATTAGATGGCATGGCTGCCTTGTCCACCAATGCAGGTTGTCAGCTCTGCCCTCTGGCTTTGGTTGTGA
TCCCAGGACCAGCAGTCCCCAGTCCTCATTGTCATGCTCAATAGCATTCGTTAGCAATATCAGGCTGCAGATGCATTCA
CCCTCACAACTGCCCTCCTGAGTGCTAATATCATCTCCCCTTAGCCAGCTGCTCAGAGGCTCCAAAAGGTTAAGCCACA
GTCCATAGTCTCAGAGCCAGGCATAGAGGAAGGCCTCCTACCGGAATCTATCTGACTCCCAGGCCCACAACATGTCCCC
TTCCCCCTCAGGCTGCTGACACTCAGGGATGGGGATCCTGTGGCTTCGTAGGGATCTCCAGAAGGCTGGCTCCAGCTTG
TCCACAGTCTCTCAGGGGCTGTCCATTCACTCAGCAATTATATATGGAGCACCACAGCCAGGCTCTGTGCTGGGCATAA
GGACACAGTCAGGAGCAGCCAGCAAGGTCCTTCCTCCCAGAATTCACAGCCTGGTCAAGAACTCTGGTTTGACTACAG
TGTCTCATAGCATTCCACAACAGACCCGTCACCCAGTGCCACATTTGTCTTTGGTGACAAGAAACCCACATTCTGAGGC
AGGCAAGGGCACATGGGTCCTAAGGCCCATGTCTCCCATTTCTCTAAAGAGACAGTCTGCAGAAATCTGAAACTCTACG
TAAGGAGTGTGGGCAGGAAGACGAACCAGGGCTAAAGACGCCGCCTCTCTGCCCTGTGGAGGGTGGCAGGCTGTCAAG
GGACAGACACCCCACACCACAGTACTTCTCCCAGAAAGTACCATGGCCGAGAGTGGAGGTGGGACAAGAAGTGGCTG
CTTTAAGCATTGCTCAAAAGAAGGGTTGCTCCAAGCTGTGCATTCTGGGAGAGAAGACCTTGCAGCCTGCTCCTGACTG
TGTCCCTACTACGCGCAGTCCAGAGCCTGGCTGTGGCGCTTCTTACATAGTTGCTGAGTGAATGAACGGCCTCTGTCTG
AGAGGCTGTGGACAAACTGGAGACAGCCTTCAGGAGATCCTTGTGGGTTTTCTGCAAGGAAATGGCTAATGTCTGCCA
AGTTCTTCCTGTGGGAAAGACGACACACTGAATGACTTCTAGATACTGTTAATCCCAGAGACTCTGAAGCGAAGCTTCT
TCGTACCCACTTGCCAGAGGAGAACCCTTGATGATTCAAGGATGTTAAGCTAAGTCTTGGCTCTTAACCACTGCCCTGA
ATGTCTCTCCTGGACACTGTGCCCCAGGCTACCAGGCATTATGTGCCTCCCACGGGCCCATCTGTTCAGCTAGCAGGAC
CCTTGTGCTTACCACAGGTCCTACTACCTGCCACCTACTTCTCAGCGACACCCAGGAAGATGGCCGGCCCGTGGATTAT
CACCCTCCTCTGTGGTTTGCTGGGAGCCACACTGGTCCAAGCTAACGTCTATCCCCCTGCAGTGCTCAACCTTGGCCCA
GAAGTCATCCAGAAACGTAAGTAATGTGCCCCGAGGCCTCAGCACCATAGAGCAAGGGGCAGATGGAGCAGTCCTCCA
AAAAGGAAGGGAGTTCCTCATCAAAGGAAAATACCAGGCAGGGGTGGAGCCCAGGGCGTGAGTGAGACAAGTAATGG
CTGATGTAAAAGTCTGGGATAGTATGATCTCAACAGCCCATGATACCAGGATGTCCTCACATAATATTATTCCAAGCAC
CTACTAGTGGCAGAATCTAGTGACTTCAGTCCCACACAAGGGTTTGTTACTCCAGACCCATGACCCTAGGCCTGGCCCT
AGACCTACGCTTATTCTTTGTAAGAACATGGGAAGGAAGGCCTTCCTCCCTGGGCCAACGGGAACTCCCAATACCATAG
AACTGAGGAGAGTCCCAGGCTCCCTAGGCAGCCTTCTCCCCAGCGCATTTCTGCCATGGGACTCAGTCCTGTGTGTTAG
AGGACACTTGGCAGCAACTTCCACCCAGGCCCTGATACCCGCCTCCTATGGCATTGTGGCAATTACTTGGTAGACCAGC
ACTGTTCCAAAGGCCAGGGGAAGCACATGGCCTAAGGCGAATCCTATGATTCCCAAGCATGTGGTATGTGAGCTATGG
GGCCCTTGGGGTAAGCGGCATCGTGAAAGTCTGCAGCCAAGGAGTTAGTACCAAAGGTAGTGTGGGAGGAGATTCCTA
GGATCCCCTGCAGGGGAGTCAGTGGGCCCACCCAAGCAAGTGACCACTGAGCACCAGGAGCCACAAAGAAACTGTATT
AATTAACTCCATTAGAAGCCGAAAGCCACACCATAAACCTGGGGTGGGATGTTTAATGTAAAGAAGAGTAATGGGACA
TCAAAGGAGAGAGGATTTGCCTAAGAGGGGAGTGATATAAAGAACTCTAAAGGGCACCCAGAAACGAGGTGGCACCC
AAGAACAGAACAAACTCAGAATCGGAGACCCCATCCCAGGCCTCATGGGCCTGGGTATGACTCAAGGTTGGGTGGAGG
AAAGCTTGCTAGGTTGTGCCAAACCAGTGCTGCCCCACTGCTGGCCAACAGACGCTGCCGCGCTCACCCCTGCCCTGAG
CCGATGATATCCTTTGGGTGATGTCCCTGGAGGGGGGTCATCTCTGGGTGGCCCCTGGAAGCTTGCCAGGAGCCCTCAA
AGTTTACTCTCAGCCTTCACGAGCTCAACATTACCCGGGCAGTGATACCTAACACAGGGGCTAAGAGGCATGCATCGG
AGACTTTCCACTCCATGTCCCTCAAAAGCCTATGCTGTTGAGGTTTAAGACTGCCAACTGGCAAAGATGAAATGTAATG
GGTCCAGCCCCATTAGCACAGAGGAGGCAAATGTTTGATAGGAATGCCATGGGGGCTCTCAAACACGGACTGGCTCGG
CTGCAGCACACTGCCTATGAAATGGGCTCTGGCCACCGGTGAAAAGCCGGCAGGGACCAGCCCCAGGGCCCTTGTTCC
CATTTGTTCCTTCCTCTGCTCAGCACCTAAGGCACTTTGGACAATAGAGCAAACATTGAGAGTGCCAGAAGATCATTAT
CCGGTGTGGCCTGGCCACAAAACTGTCCCCAGTGGAATGCTCAACTCAAGAAGTATTTGGCACTAGCGAGGGAGAAAG
AAGACAAGGGTTCTGGCGTCCCAGTGACTGGCGACATTCTGAGTGTGGTGATTCCCACTATCTCCAGCCTCACTGTCCA
CCCAGGGACACCTCCCTCCAGGGCACATCTACATGGTGAAGATGATCAGGCTGAAAAGGCAGAGCCCTCACTTCATGT
CACATCCAAGTGGCCCTCAGTGATGGCTACCCTCAACCCGGAGCCACTGCTAGGCTACTCCTCTTGGCCCTCAGTGATG
GACCTTCAACCCGGAGCCACTACTATGCTGTCCCGCCCCCCCCGCCCCCATCTTGACATCTAATCCCAGCATCTCTCTGC
AGACCTGACCCAGGCACTGAAGGACCATGATGCCACTGCCATCCTCCAGGAGTTGCCACTGCTCAGAGCCATGCAAGA
TAAGTCTGGCAGTATCCCCATACTGGACAGCTTCGTGCACACCGTTCTGAGATACATCATATGGTGAGTGGGCCCGACA
GCATTGGGAGCCATCTCTTCCCCACACCTCTGCCTACACATTGTGCAGCTGTCAATCAACAGTTATGAAGACAGACCAG
ACTAAGTCTCTCTTTTGATAAGCCCCCAGAATGGCTAGTGGTGTCCATAAGGCAGTGTCACTGGTACCTCTAAGAGGGA
AGCCACTTAGGAAGGTTCCCCAGAGTGCTGGAACCTGGAGGTGCTTTTTAATGGAGGTCATGGTTAAGCCAGGGACAC
CAAGAAACTGATGTTTCAGGCAAAGGGGCCAGTGGGGCAAAACCATGGAGGGGCAAGAATCACACAATTCGAACCCC

AACAGCAAAGGATGACAGGACAGTAGGGTCAGGTCATGGAGGAGCTCTGTCAGCCCAACAGGCTGAGGCTAGAAGCC
GAGGAACCTGAAGAGAAGCAAGCGAGGGTGAAGGGGAGAGACAGAGGTAGGGCCATGATGGAAAGGATTAGTAAAC
TAGGCTTGGGATCCAGGCTCTGCTCAGGTGTGAGGAAGTCAGGGGAAAGGATGGGAGCAGGCTTGGGGCTGACTCTCG
TCCGAGTGTGAACATGATTCTCATCCCCCTGCACCCTGTCCAGGATGAAGGTCACCTCTGCTAACATCCTCCAGCTGGA
TGTGCAGCCTTCAACTTATGACCAGGAGCTGGTGGTCAGAATCCCCCTGGACATGGTGGCTGGACTAAACACGTGAGTG
TCCCCTGAGTCAGACACAGCTAACCTAACAGTTTCAGCTACTCGCTCTCCATGCAGATGTCTAGAAACAAATCCCAGCA
TTAGATGAACAAGTGCAAAGGCCCTGAGGCAGAAATGACAACAAGAAGGGAAGGGGTGGGGTTAGAGATGAGGTCAG
CAAGCCGCTGAGGACTGATGGTGCCGGACCTTATGTGACAAGATGAGAACTAGGCTTTAATGGAATATGAGGGCCAAA
AGCAACAGAAGGTCTGAGCTTAGGGGAAAACAGTGTGGTGCAGAGTGGAAGTGGGACCACCACATGGAAGGCCCTGC
TCTAGGGGAGACAGAAGTGGCTGAG:GTAGCCAGGCAGTGGTGGCACACACCTTT:AATCC:CAGCACTTGGGAAGCAG
AGGCAGGCGGATTTCTGAGTTTGAGGCCAGCCTGGTCTACAAAGAGAGTTCCAGGACAGCCAGGGCTATACAGAGAAA
CCCTGTCTCGGGGAAAGAAATAAAAAGTGCCTAAGGCCCATGTCTGGGATGATGGAGCAGAGGAGGAAACAGCCTCTA
GCTATGAGTTGGAAGCAGGCAGAAACAACAGGGATTCCTGTGAGTTGGATATGGAGAGAGATCAAGGATGACTTCAGG
GTCTGCTCCCACAACAATCCGGGAGGAACAGCCAGCAGCCAAGACGACAAGGTTTGAAAGAGGCAGGCCAGGAGACA
ATGTGGTGTGACTTGGAATCCCCTGGCATCCATAAGTGGTGAGATCCGGAGAAAACTCTGGGACTCCAGATCTGGGAG
AGATAATGGGAACCAGCAAAAGAGATAGTGAAGGACAGATAGACTGCTGTGGCACACAGACTTCAACAGCACCCTTTC
TCACTGCTGTTGTCCCCTCAGACCACTGATCAAGACCATAGTGGAGTTCCAAATGAGCACCGAGGTCCAAGCCCTCATC
CGGGTGGAGAGGAGCAAGAGCGGCCCCGCCCACCTGAATCTCAGCGACTGCTCCAGCAATGAGAGCACCCTGCGCCTC
AGCCTGCTTCACAAGTGAGTGTTGGGCTCCTGGTCGGAGGGGCAGACCATGGCCTTGCCCACTGTACTCGTGGACAACA
CTGGCGAAACAGAAAGGCGTCACTCTGAGTCTAGATCCAGGGCTCCTTCCAGGGAATCCAGATGCCAAAGGCAGTTCT
GGGTAGCAAAAATGTAAGCAGCAATGGTGGCACCACCAGGTTGGGAGGGTGGGTATGGAGTAGATGTAAGTGACCTG
CAGACAGAGCAGCGTCTCATCCAGGGCTCCCAGACCTCCTAGGCAGGCCTAGGTGAGCCCAAAGCAAGCTCACCTTCT
CCGTTGACTGCCTAGCTGATGTTGGGCAAGTCACTTTCCCATATCTCACTTTCCCAGTACAGAAAATGCTGCTAATGACA
CCTTCCAGGCAGCACACGTGACCCACTCAAGAGTGCAATGTCACCTCCAGGGGACCTGTCAGCACCTTCCTCCTAGGAT
TCCATGTCACCGCTATAAAGCAGGGACAATCCTCTCTTACAAGCTCCCTAATCTCCCGCTCTCCTTTGCCAAAGGCTCTC
CTTCGTGGTCAACTCCTTGGCAAAGAATGTTATGAATCTCCTGGTGCCAGCCCTGCCCCAAATAGTGAAAAACCACGTG
AGTAAGACAGGCCTCTGGCCTATGGAGATGGCACCCCCTGTAGGACTAGGGTGGAACTGCAAGCTGGTACCGTATGAG
TTTTGTCTCCTTGGGTGGGATCCTTCCGGGACTGAGTAATTCCTTCAACTAAACCAAGAACCACTGAGCCAATAAACTG
AGCCACTGACCCAGCAAGATCATTGAGGTCAAAGTAACAGGAAAGTCAAAAATTCAGGAGGCCTTGATCATGTGCTGG
TCAAGGTTTTAAAACGTGTCTCTTAAAGGGTTGAGGGAAGGATCCCTAACTGTAGCTTTTGCTGACTTCCCAGTTATAA
ATCCTACCTTCAGGCTGACTTTTGTCTATAAAAGTGATGTCATGGAGCAGAGCTGGAAGAAGGTACACAGTAGCAAGCT
CCTGTGTAGTATTGCCACCCAACAGACAGGAGACAGGAGACAGTACCTCAAGTGCATCAGACAGGGGACGGTACCTCA
CGAGCATCAGACAGGAGACGGTACCTCACGAGCATCAGACAGGAGATGGTACCTCACGAGCATCAGACAGGAGTCAG
GAGCTAGTACCTCAAGTGCATTAGACAGGAGACAGGAGACAGTACCTCACGTGCATCAGACTGGAGACTGCTTTAATA
GACAGTAGTGGATGCAGAGCAAAACACCTGGGAGAGGATGGGTTTTCTGCCTTTATTAAATGTATTTTAGTATGTTTTA
TGTAAGTATATATACTTTGATTTTAAAAACCAAGCCAGAAATCCCTGAACATCCTGCATTTTGTCTTGAAATGTTGTCAT
AAGCAGACTGTGGCATGCTCAGCTGTCCAGCTGTGGTTTGAAAGCACTGCAGCCCACCAGATCCTCGCTCCGTTTATTT
CTTCTGAGTCCGTAAATTCAGGCTGTGTGTAGGGGGTTGGGGAGGATAGAACCCACCTACTTTACACCTACTAGACAAG
TGGTCTATAGCAAAAGATACCTGCAAACCTCTCATTTTATATGCCCTTAATATGCCATGGGTAAACTGAAAKKTTYAGA
AGCGGGTWCTTTCATATTGACCAGTGATTTCCGTTGTAAATTCAAGATGCTTTAGCCAGGCAATAGTGGTGTATGTCTT
TAGTTCCAGCACTTGGGAGGCAGAGACAGGCAGATCTCTGAGATTAAGGCCAGCCTGACCTACAGAGTGAGTTCCATG
ACAGCTAGGGCTACACAGAGAAACCCTGTCTCAAATTATC

Selection Cassette:

GGCGCGCCGGATCCCGGGCCGCTCTAGCTAGACTAGTCTAGCTAGAGAATTCCGCCCCCCCCCCCCCCCCC
CTCTCCCTCCCCCCCCCCTAACGTTACTGGCCGAAGCCGCTTGGAATAAGGCCGGTGTGCGTTTGTCTATAT
GTTATTTTCCACCATATTGCCGTCTTTTGGCAATGTGAGGGCCCGGAAACCTGGCCCTGTCTTCTTGACGAGC
ATTCCTAGGGGTCTTTCCCCTCTCGCCAAAGGAATGCAAGGTCTGTTGAATGTCGTGAAGGAAGCAGTTCCT
CTGGAAGCTTCTTGAAGACAAACAACGTCTGTAGCGACCCTTTGCAGGCAGCGGAACCCCCCACCTGGCGA
CAGGTGCCTCTGCGGCCAAAAGCCACGTGTATAAGATACACCTGCAAAGGCGGCACAACCCCAGTGCCACG
TTGTGAGTTGGATAGTTGTGGAAAGAGTCAAATGGCTCTCCTCAAGCGTATTCAACAAGGGGCTGAAGGAT
GCCCAGAAGGTACCCCATTGTATGGGATCTGATCTGGGGCCTCGGTGCACATGCTTTACATGTGTTTAGTCG
AGGTTAAAAAAACGTCTAGGCCCCCCGAACCACGGGGACGTGGTTTTCCTTTGAAAAACACGATGATAAGC
TTGCCACAACCATGGAAGATCCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTA
ATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCC
AACAGTTGCGCAGCCTGAATGGCGAATGGCGCTTTGCCTGGTTTCCGGCACCAGAAGCGGTGCCGGAAAGC
TGGCTGGAGTGCGATCTTCCTGAGGCCGATACTGTCGTCGTCCCCTCAAACTGGCAGATGCACGGTTACGAT

GCGCCCATCTACACCAACGTGACCTATCCCATTACGGTCAATCCGCCGTTTGTTCCCACGGAGAATCCGACG
GGTTGTTACTCGCTCACATTTAATGTTGATGAAAGCTGGCTACAGGAAGGCCAGACGCGAATTATTTTTGAT
GGCGTTAACTCGGCGTTTCATCTGTGGTGCAACGGGCGCTGGGTCGGTTACGGCCAGGACAGTCGTTTGCCG
TCTGAATTTGACCTGAGCGCATTTTTACGCGCCGGAGAAAACCGCCTCGCGGTGATGGTGCTGCGCTGGAGT
GACGGCAGTTATCTGGAAGATCAGGATATGTGGCGGATGAGCGGCATTTTCCGTGACGTCTCGTTGCTGCAT
AAACCGACTACACAAATCAGCGATTTCCATGTTGCCACTCGCTTTAATGATGATTTCAGCCGCGCTGTACTG
GAGGCTGAAGTTCAGATGTGCGGCGAGTTGCGTGACTACCTACGGGTAACAGTTTCTTTATGGCAGGGTGA
AACGCAGGTCGCCAGCGGCACCGCGCCTTTCGGCGGTGAAATTATCGATGAGCGTGGTGGTTATGCCGATC
GCGTCACACTACGTCTGAACGTCGAAAACCCGAAACTGTGGAGCGCCGAAATCCCGAATCTCTATCGTGCG
GTGGTTGAACTGCACACCGCCGACGGCACGCTGATTGAAGCAGAAGCCTGCGATGTCGGTTTCCGCGAGGT
GCGGATTGAAAATGGTCTGCTGCTGCTGAACGGCAAGCCGTTGCTGATTCGAGGCGTTAACCGTCACGAGC
ATCATCCTCTGCATGGTCAGGTCATGGATGAGCAGACGATGGTGCAGGATATCCTGCTGATGAAGCAGAAC
AACTTTAACGCCGTGCGCTGTTCGCATTATCCGAACCATCCGCTGTGGTACACGCTGTGCGACCGCTACGGC
CTGTATGTGGTGGATGAAGCCAATATTGAAACCCACGGCATGGTGCCAATGAATCGTCTGACCGATGATCC
GCGCTGGCTACCGGCGATGAGCGAACGCGTAACGCGAATGGTGCAGCGCGATCGTAATCACCCGAGTGTGA
TCATCTGGTCGCTGGGGAATGAATCAGGCCACGGCGCTAATCACGACGCGCTGTATCGCTGGATCAAATCT
GTCGATCCTTCCCGCCCGGTGCAGTATGAAGGCGGCGGAGCCGACACCACGGCCACCGATATTATTTGCCC
GATGTACGCGCGCGTGGATGAAGACCAGCCCTTCCCGGCTGTGCCGAAATGGTCCATCAAAAAATGGCTTT
CGCTACCTGGAGAGACGCGCCCGCTGATCCTTTGCGAATACGCCCACGCGATGGGTAACAGTCTTGGCGGT
TTCGCTAAATACTGGCAGGCGTTTCGTCAGTATCCCCGTTTACAGGGCGGCTTCGTCTGGGACTGGGTGGAT
CAGTCGCTGATTAAATATGATGAAAACGGCAACCCGTGGTCGGCTTACGGCGGTGATTTTGGCGATACGCC
GAACGATCGCCAGTTCTGTATGAACGGTCTGGTCTTTGCCGACCGCACGCCGCATCCAGCGCTGACGGAAG
CAAAACACCAGCAGCAGTTTTTCCAGTTCCGTTTATCCGGGCAAACCATCGAAGTGACCAGCGAATACCTGT
TCCGTCATAGCGATAACGAGCTCCTGCACTGGATGGTGGCGCTGGATGGTAAGCCGCTGGCAAGCGGTGAA
GTGCCTCTGGATGTCGCTCCACAAGGTAAACAGTTGATTGAACTGCCTGAACTACCGCAGCCGGAGAGCGC
CGGGCAACTCTGGCTCACAGTACGCGTAGTGCAACCGAACGCGACCGCATGGTCAGAAGCCGGGCACATCA
GCGCCTGGCAGCAGTGGCGTCTGGCGGAAAACCTCAGTGTGACGCTCCCCGCCGCGTCCCACGCCATCCCG
CATCTGACCACCAGCGAAATGGATTTTTGCATCGAGCTGGGTAATAAGCGTTGGCAATTTAACCGCCAGTCA
GGCTTTCTTTCACAGATGTGGATTGGCGATAAAAAACAACTGCTGACGCCGCTGCGCGATCAGTTCACCCGT
GCACCGCTGGATAACGACATTGGCGTAAGTGAAGCGACCCGCATTGACCCTAACGCCTGGGTCGAACGCTG
GAAGGCGGCGGGCCATTACCAGGCCGAAGCAGCGTTGTTGCAGTGCACGGCAGATACACTTGCTGATGCGG
TGCTGATTACGACCGCTCACGCGTGGCAGCATCAGGGGAAAACCTTATTTATCAGCCGGAAAACCTACCGG
ATTGATGGTAGTGGTCAAATGGCGATTACCGTTGATGTTGAAGTGGCGAGCGATACACCGCATCCGGCGCG
GATTGGCCTGAACTGCCAGCTGGCGCAGGTAGCAGAGCGGGTAAACTGGCTCGGATTAGGGCCGCAAGAA
AACTATCCCGACCGCCTTACTGCCGCCTGTTTTGACCGCTGGGATCTGCCATTGTCAGACATGTATACCCCG
TACGTCTTCCCGAGCGAAAACGGTCTGCGCTGCGGGACGCGCGAATTGAATTATGGCCCACACCAGTGGCG
CGGCGACTTCCAGTTCAACATCAGCCGCTACAGTCAACAGCAACTGATGGAAACCAGCCATCGCCATCTGC
TGCACGCGGAAGAAGGCACATGGCTGAATATCGACGGTTTCCATATGGGGATTGGTGGCGACGACTCCTGG
AGCCCGTCAGTATCGGCGGAATTCCAGCTGAGCGCCGGTCGCTACCATTACCAGTTGGTCTGGTGTCAAAA
ATAATAATAACCGGGCAGGCCATGTCTGCCCGTATTTCGCGTAAGGAAATCCATTATGTACTATTTAAAAAA
CACAAACTTTTGGATGTTCGGTTTATTCTTTTTCTTTTACTTTTTTATCATGGGAGCCTACTTCCCGTTTTTCC
CGATTTGGCTACATGACATCAACCATATCAGCAAAAGTGATACGGGTATTATTTTTGCCGCTATTTCTCTGTT
CTCGCTATTATTCCAACCGCTGTTTGGTCTGCTTTCTGACAAACTCGGAACTTGTTTATTGCAGCTTATAATG
GTTACAAATAAAGCAATAGCATCACAAATTTCACAAATTTAATTAAGGCCGCGGGATCGATCCCGTCGAGC
AGTGTGGTTTTCAAGAGGAAGCAAAAAGCCTCTCCACCCAGGCCTGGAATGTTTCCACCCAATGTCGAGCA
GTGTGGTTTTGCAAGAGGAAGCAAAAAGCCTCTCCACCCAGGCCTGGAATGTTTCCACCCAATGTCGAGCA
AACCCCGCCCAGCGTCTTGTCATTGGCGAATTCGAACACGCAGATGCAGTCGGGGCGGCGCGGTCCCAGGT
CCACTTCGCATATTAAGGTGACGCGTGTGGCCTCGAACACCGAGCGACCCTGCAGCCAATATGGGATCGGC
CATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTATGACTGGG
CACAACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCGCAGGGGCGCCCGGTTCTTTTTG
TCAAGACCGACCTGTCCGGTGCCCTGAATGAACTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACG
ACGGGCGTTCCTTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGA
AGTGCCGGGGCAGGATCTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAAT
GCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCGCATCGAGCGAG

CACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCA
GCCGAACTGTTCGCCAGGCTCAAGGCGCGCATGCCCGACGGCGAGGATCTCGTCGTGACCCATGGCGATGC
CTGCTTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTCATCGACTGTGGCCGGCTGGGTGTGGC
GGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGCGAATGGGCTGACC
GCTTCCTCGTGCTTTACGGTATCGCCGCTCCCGATTCGCAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTT
CTTCTGAGGGGATCGGCAATAAAAAGACAGAATAAAACGCACGGGTGTTGGGTCGTTTGTTCGGATCCGAA
TTCCTCGAGGGCGCGCC

Targeted Locus:

AAACCACACTTTCCTCCATGACCAGCCAGAGACTGTATGACCTCAGGGACACTGCCCCCTCTCTCATCACCATACAGAT
GGGGACACGACTTAACAATGGATCTCCTAGCCCTAATCCATAGTTTACCACTTCCAGATAGAAGCCAAGACAAGCGCC
CAAAGGTCACATCAATATCCCTACTGACCCTAACATTAACCAGGTGTCACCAGGGCAGAGTGGTCAGGAGTGTGTGGC
CTGCACTAGGTGAATGGGCTCAAGGCCTCTCCTTGACACCCCTGGTCCTGTGACCTTGAACAAGTCACTATCTGCTGAG
TCTCCAGTGCTCCTGGAAAAAGCAGAATTGTTAATTCCCATCTGGTGCTGTCTGGCTAAGTGTGTCCAGGCAAGGATGA
CAAGGGTCCCTCTGGCCAGTTCACTCTTGTCCCTGGGCCCATGGTTCATCGCTCATTATTCTTTACCTCCACAGACATCT
ACTGCCTTCCTGGTGGTATAAATGAAGCCCATAGCTCAGCCTAGTAGACAGCACTACCATGAACCTCCATTGTACCGAG
GAGGTTATAGAGGCCCTGAGAGGTGAAGCCCTTTGCCCAGGGAAGCACAGCAAAGAAGTATCAGAGCGGAGGAGGGG
GATTCACACCTATTTTCTTAAAGGAATCAGTGAGCAGAGATGTTGTGTCCTTGCTGCATAGAAGTGGATGTGATGGGCA
AGGCCATACCTCCCTCCCTTCCATGTGGTTGCCCTGACTAAACTGTCTAATCACTTCTCTATAAACCTCATCCTGTTGTA
AGCTGTAGCAATGGCAACAGCCCCAAACGCACTGCTACTGAGCATCCCTCCGAGTAGGCCACATCCCCAGCTATATGC
CCTGTAAGACAGAGGAGCCTGCAAATGCCCATTTCACATATGGAAACTAAGAGGCTCAGAGTCCCAAGTCACTTCGAG
TGAATAAGGTGAAGTCAGGGCTTGAACCTGCTTTGTCCCCTAACACACATCCTGCACCTACCATGCTGGTGAGATGTCC
CCAGATGGCTTTTGAAGCCAGATTATTTCTGCTTGGACTCGTGGCTCTTCCGTTTCCTGACTGTGGAGGGCAGTTCCTCC
TCACTAATAGCAGGATGACTGGCTTCCCACGCACGAGGATGATACCCACCAACCTAACTGATAGAAAACACAAGGTGA
CATCTGCCATGCTGGAGATGTTCGTCCATCGTGACTACTGCTGTGCTCCTCATAGTCCTGTAAGAGGCAGGGGGTCCTT
GTGCTCACAGATAAGCTAGGGGAACCAGGACTGTTAAACTCACAGTTGTTCCTTCAGGGAAAGAAATGCATAGCCTGT
AATCCACCCAAAAGCCTGGGAGAGGACATGTTTACTAGCATGGCGACCTTTGCTCTGTCTGTATGGCTAGAGACTTTTC
CAGTGCCAGATCCTCCCCAAGACCCTTACTGTGAATTTGTTTTCCTCAGTTAATCCATAGAAGCTATCTTTATGGACTTT
ATAAGAGTTCCAATACAGTCATGACTCATCTTCATTTAGCTTACTTTGTTCCTCGGGGAGACTTACATATGCTTTGTTGT
GGATGTGAAATTGATTTCATTATCGCCAAATAAGTTTTCACCAAATAGTGCTGTGCTTAAATATGCCTAAAATAGCCAA
CTCAGGTCAGATTTCAGAAATTTGAGCCAACGCCGGCTAGAGTCACATTGAATAGAACTGCCTTCTTGTCCCCCATGGA
TCCTCTCTCTTCTGGCTGTAGAGGTTGCAGAGACCACTACGTGGTCCTGTGATGGTGACTTTCCAGGAATCAGAGAGCC
CTTGTAAGGTTTGCTGCATGCCTGCCATATGCCTTAGTGCTCCCTCTGTCCCTCCAGCCAGCTTGGATCCTCCTTTCTCCA
AACCTCAGGAAGCCAGAGTTCTGAGGCTGCTTCCAGGAAGGGCTTTGGTTCACCAAGAGCTGCAAATATTTACCTTGGA
GACCTCAGCCCAGCCTGAGGTCTTGGCCCACACACTCAGGGTGGCTGCCCAAGTCAGGATGCAAGATATAAGGCTGGG
TCTCTAAACCCACCACCCAGGAGAGGAGCAGACAGACCCAGGACTCTGGCACATTCAGGTAAACCGTGAGGAGGGTCT
AGAAGCCTGGACAGTCGGGAAGGTTCCTCACAGGGAGGAGTCACAGAGGTTAGCCCAATTGCTGGGCGAGAATTGAGC
CTAATAATGCCTCTGTCAGCTATGGTTTCTGGGAGTCAAAGAGAGAAGCAGGGAGGTGCTGGCATTCTCATCCAAAAG
CCATAAACAAAGATGGAAGCATGAGACAGGGTGGTCTTTTCTAGTCCTCAGTCTAGGGACTTCAAACCACTCTGCACAC
AGTGGCTGTGCAATGGCTGTGTGTGCTAAGCTGTTGCTGGGGACAAAGAGAAACATCTGTCACAACAACCCTCAAAGA
ACTCCTAGGTGACCATTGTGGGCTTACTGTGTTTAAGGAAGGCCCAGTAATAGAGAAGGAGTCAGAGACGCCTTTCTGG
GAGAGATGGTCTATAAGATGGAGAGCTGAGTGGATGCTGGAGAGGAGCAAAGGCATCCATGAAAAAGGGAAAGGGGT
TGAGGACCACATAGTGGGTCCATGATGAGGACAGAGATTGTGGATCACATAGTGGGTCTAAAAAGAAGGCAGACATTG
AGGACCATGTAATGAGTCCATAATGAAGGCAGGGACTGAGGACCACATAGTAGGTCCATGAAGAGGGCAGGAGATGA
GGAATAAATGGTTGATGGGCACAGGCTGGGACTTGGGGGAGACAGTATCTCCCAGAAGAAGCAATGCTACAGGTTCTG
GGTTTAGAGCCCCTCCGATCCAGAGGCTGAGCAGGGGGCAGGAGAAGAGGACAGACAGGATCCAAAGTGTTTGGAAG
ACAAAGACACCATTAGATGGCATGGCTGCCTTGTCCACCAATGCAGGTTGTCAGCTCTGCCCTCTGGCTTTGGTTGTGA
TCCCAGGACCAGCAGTCCCCAGTCCTCATTGTCATGCTCAATAGCATTCGTTAGCAATATCAGGCTGCAGATGCATTCA
CCCTCACAACTGCCCTCCTGAGTGCTAATATCATCTCCCCTTAGCCAGCTGCTCAGAGGCTCCAAAAGGTTAAGCCACA
GTCCATAGTCTCAGAGCCAGGCATAGAGGAAGGCCTCCTACCGGAATCTATCTGACTCCCAGGCCCACAACATGTCCCC
TTCCCCCTCAGGCTGCTGACACTCAGGGATGGGGATCCTGTGGCTTCGTAGGGATCTCCAGAAGGCTGGCTCCAGCTTG
TCCACAGTCTCTCAGGGGCTGTCCATTCACTCAGCAATTATATATGGAGCACCACAGCCAGGCTCTGTGCTGGGCATAA
GGACACAGTCAGGAGCAGCCAGCAAGGTCCTTCCTCCCAGAATTCACAGCCTGGTCAAGAACTCTGGTTTGACTACAG
TGTCTCATAGCATTCCACAACAGACCCGTCACCCAGTGCCACATTTGTCTTTGGTGACAAGAAACCCACATTCTGAGGC
AGGCAAGGGCACATGGGTCCTAAGGCCCATGTCTCCCATTTCTCTAAAGAGACAGTCTGCAGAAATCTGAAACTCTACG
TAAGGAGTGTGGGCAGGAAGACGAACCAGGGCTAAAGACGCCGCCTCTCTGCCCTGTGGAGGGTGGCAGGCTGTCAAG
GGACAGACACCCCACACCACAGTACTTCTCCCAGAAAGTACCATGGCCGAGAGTGGAGGTGGGACAAGAAGTGGCTG

CTTTAAGCATTGCTCAAAAGAAGGGTTGCTCCAAGCTGTGCATTCTGGGAGAGAAGACCTTGCAGCCTGCTCCTGACTG
TGTCCCTACTACGCGCAGTCCAGAGCCTGGCTGTGGCGCTTCTTACATAGTTGCTGAGTGAATGAACGGCCTCTGTCTG
AGAGGCTGTGGACAAACTGGAGACAGCCTTCAGGAGATCCTTGTGGGTTTTCTGCAAGGAAATGGCTAATGTCTGCCA
AGTTCTTCCTGTGGGAAAGACGACACACTGAATGACTTCTAGATACTGTTAATCCCAGAGACTCTGAAGCGAAGCTTCT
TCGTACCCACTTGCCAGAGGAGAACCCTTGATGATTCAAGGATGTTAAGCTAAGTCTTGGCTCTTAACCACTGCCCTGA
ATGTCTCTCCTGGACACTGTGCCCCAGGCTACCAGGCATTATGTGCCTCCCACGGGCCCATCTGTTCAGCTAGCAGGAC
CCTTGTGCTTACCACAGGTCCTACTACCTGCCACCTACTTCTCAGCGACACCCAGGAAGATGGCCGGCCCGTGGATTAT
CACCCTCCTCTGTGGTTTGCTGGGAGCCACACTGGTCCAAGCTAACGTCTATCCCCCTGCAGTGCTCAACCTTGGCCCA
GAAGTCATCCAGAAACGTAAGTAATGTGCCCCGAGGCCTCAGCACCATAGAGCAAGGGGCAGATGGAGCAGTCCTCCA
AAAAGGAAGGGAGTTCCTCATCAAAGGAAAATACCAGGCAGGGGTGGAGCCCAGGGCGTGAGTGAGACAAGTAATGG
CTGATGTAAAAGTCTGGGATAGTATGATCTCAACAGCCCATGATACCAGGATGTCCTCACATAATATTATTCCAAGCAC
CTACTAGTGGCAGAATCTAGTGACTTCAGTCCCACACAAGGGTTTGTTACTCCAGACCCATGACCCTAGGCCTGGCCCT
AGACCTACGCTTATTCTTTGTAAGAACATGGGAAGGAAGGCCTTCCTCCCTGGGCCAACGGGAACTCCCAATACCATAG
AACTGAGGAGAGTCCCAGGCTCCCTAGGCAGCCTTCTCCCCAGCGCATTTCTGCCATGGGACTCAGTCCTGTGTGTTAG
AGGACACTTGGCAGCAACTTCCACCCAGGCCCTGATACCCGCCTCCTATGGCATTGTGGCAATTACTTGGTAGACCAGC
ACTGTTCCAAAGGCCAGGGGAAGCACATGGCCTAAGGCGAATCCTATGATTCCCAAGCATGTGGTATGTGAGCTATGG
GGCCCTTGGGGTAAGCGGCATCGTGAAAGTCTGCAGCCAAGGAGTTAGTACCAAAGGTAGTGTGGGAGGAGATTCCTA
GGATCCCCTGCAGGGGAGTCAGTGGGCCCACCCAAGCAAGTGACCACTGAGCACCAGGAGCCACAAAGAAACTGTATT
AATTAACTCCATTAGAAGCCGAAAGCCACACCATAAACCTGGGGTGGGATGTTTAATGTAAAGAAGAGTAATGGGACA
TCAAAGGAGAGAGGATTTGCCTAAGAGGGGAGTGATATAAAGAACTCTAAAGGGCACCCAGAAACGAGGTGGCACCC
AAGAACAGAACAAACTCAGAATCGGAGACCCCATCCCAGGCCTCATGGGCCTGGGTATGACTCAAGGTTGGGTGGAGG
AAAGCTTGCTAGGTTGTGCCAAACCAGTGCTGCCCCACTGCTGGCCAACAGACGCTGCCGCGCTCACCCCTGCCCTGAG
CCGATGATATCCTTTGGGTGATGTCCCTGGAGGGGGGTCATCTCTGGGTGGCCCCTGGAAGCTTGCCAGGAGCCCTCAA
AGTTTACTCTCAGCCTTCACGAGCTCAACATTACCCGGGCAGTGATACCTAACACAGGGGCTAAGAGGCATGCATCGG
AGACTTTCCACTCCATGTCCCTCAAAAGCCTATGCTGTTGAGGTTTAAGACTGCCAACTGGCAAAGATGAAATGTAATG
GGTCCAGCCCCATTAGCACAGAGGAGGCAAATGTTTGATAGGAATGCCATGGGGGCTCTCAAACACGGACTGGCTCGG
CTGCAGCACACTGCCTATGAAATGGGCTCTGGCCACCGGTGAAAAGCCGGCAGGGACCAGCCCCAGGGCCCTTGTTCC
CATTTGTTCCTTCCTCTGCTCAGCACCTAAGGCACTTTGGACAATAGAGCAAACATTGAGAGTGCCAGAAGATCATTAT
CCGGTGTGGCCTGGCCACAAAACTGTCCCCAGTGGAATGCTCAACTCAAGAAGTATTTGGCACTAGCGAGGGAGAAAG
AAGACAAGGGTTCTGGCGTCCCAGTGACTGGCGACATTCTGAGTGTGGTGATTCCCACTATCTCCAGCCTCACTGTCCA
CCCAGGGACACCTCCCTCCAGGGCACATCTACATGGTGAAGATGATCAGGCTGAAAAGGCAGAGCCCTCACTTCATGT
CACATCCAAGTGGCCCTCAGTGATGGCTACCCTCAACCCGGAGCCACTGCTAGGCTACTCCTCTTGGCCCTCAGTGATG
GACCTTCAACCCGGAGCCACTACTATGCTGTCCCGCCCCCCCCGCCCCCATCTTGACATCTAATCCCAGCATCTCTCTGC
AGACCTGACCCAGGCGCGCCGGATCCCGGGCCGCTCTAGCTAGACTAGTCTAGCTAGAGAATTCCGCCCCCCCCCCCCC
CCCCCCTCTCCCTCCCCCCCCCCTAACGTTACTGGCCGAAGCCGCTTGGAATAAGGCCGGTGTGCGTTTGTCTATATGTT
ATTTTCCACCATATTGCCGTCTTTTGGCAATGTGAGGGCCCGGAAACCTGGCCCTGTCTTCTTGACGAGCATTCCTAGGG
GTCTTTCCCCTCTCGCCAAAGGAATGCAAGGTCTGTTGAATGTCGTGAAGGAAGCAGTTCCTCTGGAAGCTTCTTGAAG
ACAAACAACGTCTGTAGCGACCCTTTGCAGGCAGCGGAACCCCCCACCTGGCGACAGGTGCCTCTGCGGCCAAAAGCC
ACGTGTATAAGATACACCTGCAAAGGCGGCACAACCCCAGTGCCACGTTGTGAGTTGGATAGTTGTGGAAAGAGTCAA
ATGGCTCTCCTCAAGCGTATTCAACAAGGGGCTGAAGGATGCCCAGAAGGTACCCCATTGTATGGGATCTGATCTGGG
GCCTCGGTGCACATGCTTTACATGTGTTTAGTCGAGGTTAAAAAAACGTCTAGGCCCCCCGAACCACGGGGACGTGGTT
TTCCTTTGAAAAACACGATGATAAGCTTGCCACAACCATGGAAGATCCCGTCGTTTTACAACGTCGTGACTGGGAAAAC
CCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCG
ATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCTTTGCCTGGTTTCCGGCACCAGAAGCGGTGCCGGA
AAGCTGGCTGGAGTGCGATCTTCCTGAGGCCGATACTGTCGTCGTCCCCTCAAACTGGCAGATGCACGGTTACGATGCG
CCCATCTACACCAACGTGACCTATCCCATTACGGTCAATCCGCCGTTTGTTCCCACGGAGAATCCGACGGGTTGTTACT
CGCTCACATTTAATGTTGATGAAAGCTGGCTACAGGAAGGCCAGACGCGAATTATTTTTGATGGCGTTAACTCGGCGTT
TCATCTGTGGTGCAACGGGCGCTGGGTCGGTTACGGCCAGGACAGTCGTTTGCCGTCTGAATTTGACCTGAGCGCATTT
TTACGCGCCGGAGAAAACCGCCTCGCGGTGATGGTGCTGCGCTGGAGTGACGGCAGTTATCTGGAAGATCAGGATATG
TGGCGGATGAGCGGCATTTTCCGTGACGTCTCGTTGCTGCATAAACCGACTACACAAATCAGCGATTTCCATGTTGCCA
CTCGCTTTAATGATGATTTCAGCCGCGCTGTACTGGAGGCTGAAGTTCAGATGTGCGGCGAGTTGCGTGACTACCTACG
GGTAACAGTTTCTTTATGGCAGGGTGAAACGCAGGTCGCCAGCGGCACCGCGCCTTTCGGCGGTGAAATTATCGATGA
GCGTGGTGGTTATGCCGATCGCGTCACACTACGTCTGAACGTCGAAAACCCGAAACTGTGGAGCGCCGAAATCCCGAA
TCTCTATCGTGCGGTGGTTGAACTGCACACCGCCGACGGCACGCTGATTGAAGCAGAAGCCTGCGATGTCGGTTTCCGC
GAGGTGCGGATTGAAAATGGTCTGCTGCTGCTGAACGGCAAGCCGTTGCTGATTCGAGGCGTTAACCGTCACGAGCAT
CATCCTCTGCATGGTCAGGTCATGGATGAGCAGACGATGGTGCAGGATATCCTGCTGATGAAGCAGAACAACTTTAAC
GCCGTGCGCTGTTCGCATTATCCGAACCATCCGCTGTGGTACACGCTGTGCGACCGCTACGGCCTGTATGTGGTGGATG
AAGCCAATATTGAAACCCACGGCATGGTGCCAATGAATCGTCTGACCGATGATCCGCGCTGGCTACCGGCGATGAGCG
AACGCGTAACGCGAATGGTGCAGCGCGATCGTAATCACCCGAGTGTGATCATCTGGTCGCTGGGGAATGAATCAGGCC

ACGGCGCTAATCACGACGCGCTGTATCGCTGGATCAAATCTGTCGATCCTTCCCGCCCGGTGCAGTATGAAGGCGGCGG
AGCCGACACCACGGCCACCGATATTATTTGCCCGATGTACGCGCGCGTGGATGAAGACCAGCCCTTCCCGGCTGTGCCG
AAATGGTCCATCAAAAAATGGCTTTCGCTACCTGGAGAGACGCGCCCGCTGATCCTTTGCGAATACGCCCACGCGATG
GGTAACAGTCTTGGCGGTTTCGCTAAATACTGGCAGGCGTTTCGTCAGTATCCCCGTTTACAGGGCGGCTTCGTCTGGG
ACTGGGTGGATCAGTCGCTGATTAAATATGATGAAAACGGCAACCCGTGGTCGGCTTACGGCGGTGATTTTGGCGATAC
GCCGAACGATCGCCAGTTCTGTATGAACGGTCTGGTCTTTGCCGACCGCACGCCGCATCCAGCGCTGACGGAAGCAAA
ACACCAGCAGCAGTTTTTCCAGTTCCGTTTATCCGGGCAAACCATCGAAGTGACCAGCGAATACCTGTTCCGTCATAGC
GATAACGAGCTCCTGCACTGGATGGTGGCGCTGGATGGTAAGCCGCTGGCAAGCGGTGAAGTGCCTCTGGATGTCGCT
CCACAAGGTAAACAGTTGATTGAACTGCCTGAACTACCGCAGCCGGAGAGCGCCGGGCAACTCTGGCTCACAGTACGC
GTAGTGCAACCGAACGCGACCGCATGGTCAGAAGCCGGGCACATCAGCGCCTGGCAGCAGTGGCGTCTGGCGGAAAA
CCTCAGTGTGACGCTCCCCGCCGCGTCCCACGCCATCCCGCATCTGACCACCAGCGAAATGGATTTTTGCATCGAGCTG
GGTAATAAGCGTTGGCAATTTAACCGCCAGTCAGGCTTTCTTTCACAGATGTGGATTGGCGATAAAAAACAACTGCTGA
CGCCGCTGCGCGATCAGTTCACCCGTGCACCGCTGGATAACGACATTGGCGTAAGTGAAGCGACCCGCATTGACCCTA
ACGCCTGGGTCGAACGCTGGAAGGCGGCGGGCCATTACCAGGCCGAAGCAGCGTTGTTGCAGTGCACGGCAGATACAC
TTGCTGATGCGGTGCTGATTACGACCGCTCACGCGTGGCAGCATCAGGGGAAAACCTTATTTATCAGCCGGAAAACCTA
CCGGATTGATGGTAGTGGTCAAATGGCGATTACCGTTGATGTTGAAGTGGCGAGCGATACACCGCATCCGGCGCGGAT
TGGCCTGAACTGCCAGCTGGCGCAGGTAGCAGAGCGGGTAAACTGGCTCGGATTAGGGCCGCAAGAAAACTATCCCGA
CCGCCTTACTGCCGCCTGTTTTGACCGCTGGGATCTGCCATTGTCAGACATGTATACCCCGTACGTCTTCCCGAGCGAAA
ACGGTCTGCGCTGCGGGACGCGCGAATTGAATTATGGCCCACACCAGTGGCGCGGCGACTTCCAGTTCAACATCAGCC
GCTACAGTCAACAGCAACTGATGGAAACCAGCCATCGCCATCTGCTGCACGCGGAAGAAGGCACATGGCTGAATATCG
ACGGTTTCCATATGGGGATTGGTGGCGACGACTCCTGGAGCCCGTCAGTATCGGCGGAATTCCAGCTGAGCGCCGGTCG
CTACCATTACCAGTTGGTCTGGTGTCAAAAATAATAATAACCGGGCAGGCCATGTCTGCCCGTATTTCGCGTAAGGAAA
TCCATTATGTACTATTTAAAAAACACAAACTTTTGGATGTTCGGTTTATTCTTTTTCTTTTACTTTTTTATCATGGGAGCC
TACTTCCCGTTTTTCCCGATTTGGCTACATGACATCAACCATATCAGCAAAAGTGATACGGGTATTATTTTTGCCGCTAT
TTCTCTGTTCTCGCTATTATTCCAACCGCTGTTTGGTCTGCTTTCTGACAAACTCGGAACTTGTTTATTGCAGCTTATAAT
GGTTACAAATAAAGCAATAGCATCACAAATTTCACAAATTTAATTAAGGCCGCGGGATCGATCCCGTCGAGCAGTGTG
GTTTTCAAGAGGAAGCAAAAAGCCTCTCCACCCAGGCCTGGAATGTTTCCACCCAATGTCGAGCAGTGTGGTTTTGCAA
GAGGAAGCAAAAAGCCTCTCCACCCAGGCCTGGAATGTTTCCACCCAATGTCGAGCAAACCCCGCCCAGCGTCTTGTC
ATTGGCGAATTCGAACACGCAGATGCAGTCGGGGCGGCGCGGTCCCAGGTCCACTTCGCATATTAAGGTGACGCGTGT
GGCCTCGAACACCGAGCGACCCTGCAGCCAATATGGGATCGGCCATTGAACAAGATGGATTGCACGCAGGTTCTCCGG
CCGCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCT
GTCAGCGCAGGGGCGCCCGGTTCTTTTTGTCAAGACCGACCTGTCCGGTGCCCTGAATGAACTGCAGGACGAGGCAGC
GCGGCTATCGTGGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTG
GCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCT
GATGCAATGCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCGCATCGAGCGA
GCACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAA
CTGTTCGCCAGGCTCAAGGCGCGCATGCCCGACGGCGAGGATCTCGTCGTGACCCATGGCGATGCCTGCTTGCCGAATA
TCATGGTGGAAAATGGCCGCTTTTCTGGATTCATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGC
GTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGCGAATGGGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCT
CCCGATTCGCAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAGGGGATCGGCAATAAAAAGACAGAATAA
AACGCACGGGTGTTGGGTCGTTTGTTCGGATCCGAATTCCTCGAGGGCGCGCCGCAGACCATGGCCTTGCCCACTGTAC
TCGTGGACAACACTGGCGAAACAGAAAGGCGTCACTCTGAGTCTAGATCCAGGGCTCCTTCCAGGGAATCCAGATGCC
AAAGGCAGTTCTGGGTAGCAAAAATGTAAGCAGCAATGGTGGCACCACCAGGTTGGGAGGGTGGGTATGGAGTAGAT
GTAAGTGACCTGCAGACAGAGCAGCGTCTCATCCAGGGCTCCCAGACCTCCTAGGCAGGCCTAGGTGAGCCCAAAGCA
AGCTCACCTTCTCCGTTGACTGCCTAGCTGATGTTGGGCAAGTCACTTTCCCATATCTCACTTTCCCAGTACAGAAAATG
CTGCTAATGACACCTTCCAGGCAGCACACGTGACCCACTCAAGAGTGCAATGTCACCTCCAGGGGACCTGTCAGCACCT
TCCTCCTAGGATTCCATGTCACCGCTATAAAGCAGGGACAATCCTCTCTTACAAGCTCCCTAATCTCCCGCTCTCCTTTG
CCAAAGGCTCTCCTTCGTGGTCAACTCCTTGGCAAAGAATGTTATGAATCTCCTGGTGCCAGCCCTGCCCCAAATAGTG
AAAAACCACGTGAGTAAGACAGGCCTCTGGCCTATGGAGATGGCACCCCCTGTAGGACTAGGGTGGAACTGCAAGCTG
GTACCGTATGAGTTTTGTCTCCTTGGGTGGGATCCTTCCGGGACTGAGTAATTCCTTCAACTAAACCAAGAACCACTGA
GCCAATAAACTGAGCCACTGACCCAGCAAGATCATTGAGGTCAAAGTAACAGGAAAGTCAAAAATTCAGGAGGCCTTG
ATCATGTGCTGGTCAAGGTTTTAAAACGTGTCTCTTAAAGGGTTGAGGGAAGGATCCCTAACTGTAGCTTTTGCTGACT
TCCCAGTTATAAATCCTACCTTCAGGCTGACTTTTGTCTATAAAAGTGATGTCATGGAGCAGAGCTGGAAGAAGGTACA
CAGTAGCAAGCTCCTGTGTAGTATTGCCACCCAACAGACAGGAGACAGGAGACAGTACCTCAAGTGCATCAGACAGGG
GACGGTACCTCACGAGCATCAGACAGGAGACGGTACCTCACGAGCATCAGACAGGAGATGGTACCTCACGAGCATCA
GACAGGAGTCAGGAGCTAGTACCTCAAGTGCATTAGACAGGAGACAGGAGACAGTACCTCACGTGCATCAGACTGGA
GACTGCTTTAATAGACAGTAGTGGATGCAGAGCAAAACACCTGGGAGAGGATGGGTTTTCTGCCTTTATTAAATGTATT
TTAGTATGTTTTATGTAAGTATATATACTTTGATTTTAAAAACCAAGCCAGAAATCCCTGAACATCCTGCATTTTGTCTT
GAAATGTTGTCATAAGCAGACTGTGGCATGCTCAGCTGTCCAGCTGTGGTTTGAAAGCACTGCAGCCCACCAGATCCTC

GCTCCGTTTATTTCTTCTGAGTCCGTAAATTCAGGCTGTGTGTAGGGGGTTGGGGAGGATAGAACCCACCTACTTTACA
CCTACTAGACAAGTGGTCTATAGCAAAAGATACCTGCAAACCTCTCATTTTATATGCCCTTAATATGCCATGGGTAAAC
TGAAAKKTTYAGAAGCGGGTWCTTTCATATTGACCAGTGATTTCCGTTGTAAATTCAAGATGCTTTAGCCAGGCAATAG
TGGTGTATGTCTTTAGTTCCAGCACTTGGGAGGCAGAGACAGGCAGATCTCTGAGATTAAGGCCAGCCTGACCTACAGA
GTGAGTTCCATGACAGCTAGGGCTACACAGAGAAACCCTGTCTCAAATTATC