Supplementary Information: "Rationally Designed DNA Looping Peptides Control DNA Topology," by Gowetski, Kodis, and Kahn. Nucleic Acids Research, 2013.

Additional files provided in a zip archive: PDB files for models of LZD73 and LZD87 bound to DNA, PDB files for models of minicircle topoisomers formed from DNA loops, Microsoft Excel spreadsheet with the topoisomer distributions for the experiment of Figure 4.

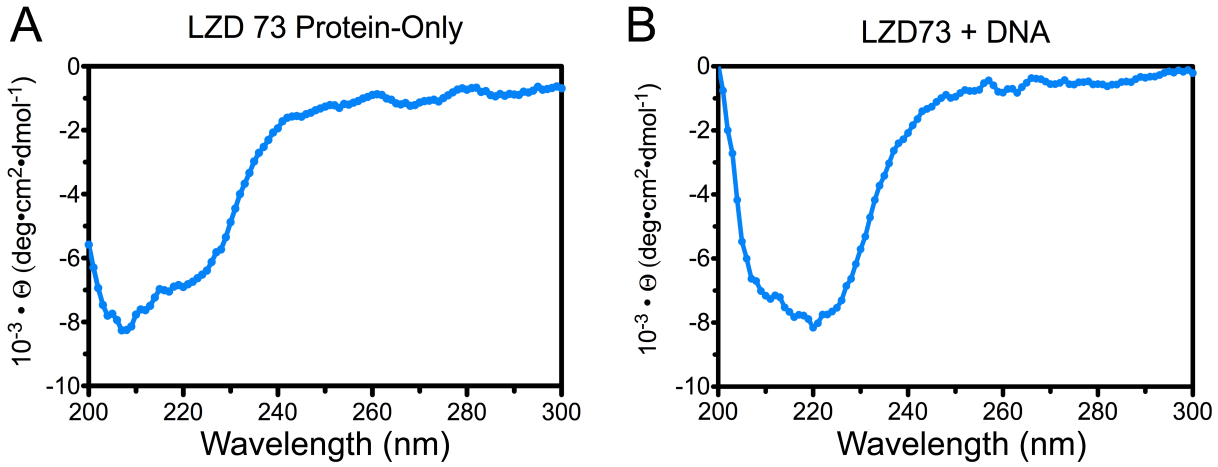
SI Materials and Methods:

Protein Expression and Purification

The gene for LZD73 was synthesized commercially (BioMatik Inc.) and was cloned into pRSET A (Invitrogen/Life Technologies) using BamHI and EcoRI restriction sites. The gene for LZD87 was cloned using standard methods by inserting an additional 42 bp (coding for 14 aa, two coiled coil turns) into the plasmid containing *Lzd73*. The peptides were expressed in *E. coli* BL21 DE3 (pLysS) cells, grown in LB media with ampicillin (100 μg/ml) and chloramphenicol (40 μg/ml) at 37 °C, induced with 0.5 mM IPTG during a 14 hour induction. Cells were lysed by French press in 10 mM MES pH 6.0, 0.5 M NaCl, 20 mM imidazole. The peptides were purified from the soluble supernatant by FPLC with a Co^{2+}-charged HiTrap chelating affinity column (GE Healthcare) utilizing a 20-400 mM imidazole gradient. The peak elution fractions were concentrated using a Centricon Ultra 4000 (Millipore), and were buffer exchanged into a storage buffer of 10 mM Tris HCl pH 7.7, 150 mM NaCl, and 10 % glycerol using Biospin 6 columns (BioRad). The proteins were quantitated by UV absorption using an estimated ε(280 nm) = 8480 $M^{-1}cm^{-1}$ from the ProtParam tool (ExPASy). Peptide molecular weight and purity (est. > 98 % based on Coomassie staining) was confirmed using SDS PAGE.

The expected α-helical folding of the coiled-coil was verified by circular dichroism (CD) as shown in Fig. S1.

Supplementary Figure S1: CD analysis of the LZD73 peptide. (A) LZD73 peptide alone (1.2 μM) in 20 mM Tris-HCl (pH 8.0 @ 25°C), 50 mM NaCl, 2 mM $CaCl_2$. (B) LZD73 (1.2 μM) with 58mer CREB site dsDNA (1 μM). The two dips at 210 nm and 220 nm are indicative of α-helical folding. An increase in the amplitude of the 220 nm signal is observed upon DNA addition, consistent with additional folding of the basic region upon DNA binding.



DNA Sequences Used in Cyclization Assays

The DNA fragments below were made by PCR, subjected to XhoI digestion, and gel purified as described in the text. Each sequence contains one CREB site (5′-ATGACGTCAT-3′) and one Inv-2 site (5′ -GTCATATGAC-3′), which are highlighted, and two XhoI sites (5′ -CTCGAG-3′), which are underlined.

Vx(448)212
AATGTGTGCCTGGCGATCTCGAGTGCAGCTGGAAGACAAGGTGGAGGAACTGCTGAGCAAGAAC
TACCACCTGGAGAACGAAGTTGCGCGCCTGAAGAAGCTGGTGGGTGAACTGCAGATGACGTCAT
GCGCGCGGATCCGAATTCTCCGGATCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTC
CCAACAGTTGCGCAGCCTGAATGGCGAATGGGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCG
GGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCG
CTTTCTTCCCTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCT
CCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAAACTTGATTAGGGTGAT
GGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGT
TCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGCGTCATATGAC
CAAGCTTGATCCGGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAG
CAATAACTAGCATAACCCCTTTTGCAGCTTTTCTCGAGTCAAGACCCGTTTAGAGGCCCC

Vx(153)414
AATGTGTGCCTGGCGATCTCGAGGAGGATAAGGATCGATGGGGATCCGATCCGCTGCTCTGAAG
CGAGCTCGGAACACTGAAGCTGCTCGACGGAGCCGAGCTCGGAAGCTGCAACGAATGAAGCAGC
TGGAAGACAAGGTGGAGGAACTGCTGAGCAAGAACTACCACCTGGAGAACGAAGTTGCGCGCCT
GAAGAAGCTGGTGGGTGAACTGCAGATGACGTCATGCGCGCGGATCCGAATTCTCCGGATCTGG
CGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAAT
GGGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGGTCATATGACCAAG
CTGAATTCGCGCGCTGACCTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAAT
ACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAA
AGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCC
TTCCTGTTTCTCGAGTTGCAGCTTTT

Vx(202)414
AATGTGTGCCTGGCGATCTCGAGGAGGATAAGGATCGATGGGGATCCGATCCAGCTGCTCTGAA
GCGAGCTCGGAACACTGAAGCTGCTCGACGGAGCCGAGCTCGGAAGCTGCAACGAATGAAGCAG
CTGGAAGACAAGGTGGAGGAACTGCTGAGCAAGAACTACCACCTGGAGAACGAAGTTGCGCGCC
TGAAGAAGCTGGTGGGTGAACTGCAGATGACGTCATGCGCGCGGATCCGAATTCTCCGGATCTG
GCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAA
TGGGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCG
CTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGGTCATATGACCAAGCTGAATTCGCGCGC
TGACCTCGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTAT
CCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTA
TTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTCTCGAGT
TGCAGCTTTT

Vx(254)414
AATGTGTGCCTGGCGATCTCGAGGAGGATAAGGATCGATGGGGATCCGATCCAGCTGCTCTGAA
GCGAGCTCGGAACACTGAAGCTGCTCGACGGAGCCGAGCTCGGAAGCTGCAACGAATGAAGCAG

CTGGAAGACAAGGTGGAGGAACTGCTGAGCAAGAACTACCACCTGGAGAACGAAGTTGCGCGCC
TGAAGAAGCTGGTGGGTGAACTGCAGATGACGTCATGCGCGCGGATCCGAATTCTCCGGATCTG
GCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAA
TGGGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCG
CTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTT
CGCCGGCTTTCCCCGTCAACGTCCGTCATATGACCAAGCTGAATTCGCGCGCTGACCTCGGAAA
TGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGA
CAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCC
GTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTCTCGAGTTGCAGCTTTT

Vx(310)414
AATGTGTGCCTGGCGATCTCGAGGAGGATAAGGATCGATGGGGATCCGATCCAGCTGCTCTGAA
GCGAGCTCGGAACACTGAAGCTGCTCGACGGAGCCGAGCTCGGAAGCTGCAACGAATGAAGCAG
CTGGAAGACAAGGTGGAGGAACTGCTGAGCAAGAACTACCACCTGGAGAACGAAGTTGCGCGCC
TGAAGAAGCTGGTGGGTGAACTGCAGATGACGTCATGCGCGCGGATCCGAATTCTCCGGATCTG
GCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAA
TGGGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCG
CTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTT
CGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTA
CGGCACCTCGACCCCTGTCATATGACCAAGCTGAATTCGCGCGCTGACCTCGGAAATGTGCGCG
GAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACC
CTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCC
CTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTCTCGAGTTGCAGCTTTT

Vx(376)414
AATGTGTGCCTGGCGATCTCGAGGAGGATAAGGATCGATGGGGATCCGATCCAGCTGCTCTGAA
GCGAGCTCGGAACACTGAAGCTGCTCGACGGAGCCGAGCTCGGAAGCTGCAACGAATGAAGCAG
CTGGAAGACAAGGTGGAGGAACTGCTGAGCAAGAACTACCACCTGGAGAACGAAGTTGCGCGCC
TGAAGAAGCTGGTGGGTGAACTGCAGATGACGTCATGCGCGCGGATCCGAATTCTCCGGATCTG
GCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAA
TGGGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCG
CTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTT
CGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTA
CGGCACCTCGACCCCAAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGAT
AGACGGTTTTTCGCCCCTGTCATATGACCAAGCTGAATTCGCGCGCTGACCTCGGAAATGTGCG
CGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAA
CCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCG
CCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTCTCGAGTTGCAGCTTTT

Vx(448)414
AATGTGTGCCTGGCGATCTCGAGGAGGATAAGGATCGATGGGGATCCGATCCGACGATAAGGAT
CGATGGGGATCCGATCCAGCTGCTCTGAAGCGAGCTCGGAACACTGAAGCTGCTCGACGGAGCC
GAGCTCGGAAGCTGCAACGAATGAAGCAGCTGGAAGACAAGGTGGAGGAACTGCTGAGCAAGAA
CTACCACCTGGAGAACGAAGTTGCGCGCCTGAAGAAGCTGGTGGGTGAACTGCAGATGACGTCA
TGCGCGCGGATCCGAATTCTCCGGATCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTT
CCCAACAGTTGCGCAGCCTGAATGGCGAATGGGACGCGCCCTGTAGCGGCGCATTAAGCGCGGC
GGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTC

GCTTTCTTCCCTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGC
TCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAAACTTGATTAGGGTGA
TGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACG
TTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGCGTCATATGA
CCAAGCTGAATTCGCGCGCTGACCTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTC
TAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATT
GAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATT
TTGCCTTCCTGTTTCTCGAGTTGCAGCTTTT

Vx(435)414
AATGTGTGCCTGGCGATCTCGAGGAGGATAAGGATCGATGGGGATCCGATCCAGCTGCTCTGAA
GCGAGCTCGGAACACTGAAGCTGCTCGACGGAGCCGAGCTCGGAAGCTGCAACGAATGAAGCAG
CTGGAAGACAAGGTGGAGGAACTGCTGAGCAAGAACTACCACCTGGAGAACGAAGTTGCGCGCC
TGAAGAAGCTGGTGGGTGAACTGCAGATGACGTCATGCGCGCGGATCCGAATTCTCCGGATCTG
GCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAA
TGGGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCG
CTACACTTGCCAGCGCCCTATCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTT
CGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTA
CGGCACCTCGACCCCAAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGAT
AGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAAC
TGGAACAACACTCGTCATATGACAACCCTATAAGCTTAAGCTGAATTCGCGCGCTGACCTCGGA
AATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGA
GACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTT
CCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTCTCGAGTTGCAGCTTTT

Vx(438)414
AATGTGTGCCTGGCGATCTCGAGGAGGATAAGGATCGATGGGGATCCGATCCAGCTGCTCTGAA
GCGAGCTCGGAACACTGAAGCTGCTCGACGGAGCCGAGCTCGGAAGCTGCAACGAATGAAGCAG
CTGGAAGACAAGGTGGAGGAACTGCTGAGCAAGAACTACCACCTGGAGAACGAAGTTGCGCGCC
TGAAGAAGCTGGTGGGTGAACTGCAGATGACGTCATGCGCGCGGATCCGAATTCTCCGGATCTG
GCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAA
TGGGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCG
CTACACTTGCCAGCGCCCTATCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTT
CGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTA
CGGCACCTCGACCCCAAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGAT
AGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAAC
TGGAACAACACTCAACGTCATATGACCCTATAAGCTTAAGCTGAATTCGCGCGCTGACCTCGGA
AATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGA
GACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTT
CCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTCTCGAGTTGCAGCTTTT

Vx(440)414
AATGTGTGCCTGGCGATCTCGAGGAGGATAAGGATCGATGGGGATCCGATCCAGCTGCTCTGAA
GCGAGCTCGGAACACTGAAGCTGCTCGACGGAGCCGAGCTCGGAAGCTGCAACGAATGAAGCAG
CTGGAAGACAAGGTGGAGGAACTGCTGAGCAAGAACTACCACCTGGAGAACGAAGTTGCGCGCC
TGAAGAAGCTGGTGGGTGAACTGCAGATGACGTCATGCGCGCGGATCCGAATTCTCCGGATCTG
GCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAA

TGGGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCG
CTACACTTGCCAGCGCCCTATCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTT
CGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTA
CGGCACCTCGACCCCAAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGAT
AGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAAC
TGGAACAACACTCAACCCGTCATATGACTATAAGCTTAAGCTGAATTCGCGCGCTGACCTCGGA
AATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGA
GACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTT
CCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTCTCGAGTTGCAGCTTTT

Vx(443)414
AATGTGTGCCTGGCGATCTCGAGGAGGATAAGGATCGATGGGGATCCGATCCAGCTGCTCTGAA
GCGAGCTCGGAACACTGAAGCTGCTCGACGGAGCCGAGCTCGGAAGCTGCAACGAATGAAGCAG
CTGGAAGACAAGGTGGAGGAACTGCTGAGCAAGAACTACCACCTGGAGAACGAAGTTGCGCGCC
TGAAGAAGCTGGTGGGTGAACTGCAGATGACGTCATGCGCGCGGATCCGAATTCTCCGGATCTG
GCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAA
TGGGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCG
CTACACTTGCCAGCGCCCTATCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTT
CGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTA
CGGCACCTCGACCCCAAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGAT
AGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAAC
TGGAACAACACTCAACCCTATGTCATATGACAAGCTTAAGCTGAATTCGCGCGCTGACCTCGGA
AATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGA
GACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTT
CCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTCTCGAGTTGCAGCTTTT

Vx(445)414
AATGTGTGCCTGGCGATCTCGAGGAGGATAAGGATCGATGGGGATCCGATCCAGCTGCTCTGAA
GCGAGCTCGGAACACTGAAGCTGCTCGACGGAGCCGAGCTCGGAAGCTGCAACGAATGAAGCAG
CTGGAAGACAAGGTGGAGGAACTGCTGAGCAAGAACTACCACCTGGAGAACGAAGTTGCGCGCC
TGAAGAAGCTGGTGGGTGAACTGCAGATGACGTCATGCGCGCGGATCCGAATTCTCCGGATCTG
GCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAA
TGGGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCG
CTACACTTGCCAGCGCCCTATCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTT
CGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTA
CGGCACCTCGACCCCAAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGAT
AGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAAC
TGGAACAACACTCAACCCTATAAGTCATATGACGCTTAAGCTGAATTCGCGCGCTGACCTCGGA
AATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGA
GACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTT
CCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTCTCGAGTTGCAGCTTTT

Vx(450)414
AATGTGTGCCTGGCGATCTCGAGGAGGATAAGGATCGATGGGGATCCGATCCAGCTGCTCTGAA
GCGAGCTCGGAACACTGAAGCTGCTCGACGGAGCCGAGCTCGGAAGCTGCAACGAATGAAGCAG
CTGGAAGACAAGGTGGAGGAACTGCTGAGCAAGAACTACCACCTGGAGAACGAAGTTGCGCGCC
TGAAGAAGCTGGTGGGTGAACTGCAGATGACGTCATGCGCGCGGATCCGAATTCTCCGGATCTG

GCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAA
TGGGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCG
CTACACTTGCCAGCGCCCTATCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTT
CGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTA
CGGCACCTCGACCCCAAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGAT
AGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAAC
TGGAACAACACTCAACCCTATAAGCTTAGTCATATGACAGCTGAATTCGCGCGCTGACCTCGGA
AATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGA
GACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTT
CCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTCTCGAGTTGCAGCTTTT

Vx(453)414
AATGTGTGCCTGGCGATCTCGAGGAGGATAAGGATCGATGGGGATCCGATCCAGCTGCTCTGAA
GCGAGCTCGGAACACTGAAGCTGCTCGACGGAGCCGAGCTCGGAAGCTGCAACGAATGAAGCAG
CTGGAAGACAAGGTGGAGGAACTGCTGAGCAAGAACTACCACCTGGAGAACGAAGTTGCGCGCC
TGAAGAAGCTGGTGGGTGAACTGCAGATGACGTCATGCGCGCGGATCCGAATTCTCCGGATCTG
GCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAA
TGGGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCG
CTACACTTGCCAGCGCCCTATCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTT
CGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTA
CGGCACCTCGACCCCAAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGAT
AGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAAC
TGGAACAACACTCAACCCTATAAGCTTAAGCGTCATATGACTGAATTCGCGCGCTGACCTCGGA
AATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGA
GACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTT
CCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTCTCGAGTTGCAGCTTTT

Vx(455)414
AATGTGTGCCTGGCGATCTCGAGGAGGATAAGGATCGATGGGGATCCGATCCAGCTGCTCTGAA
GCGAGCTCGGAACACTGAAGCTGCTCGACGGAGCCGAGCTCGGAAGCTGCAACGAATGAAGCAG
CTGGAAGACAAGGTGGAGGAACTGCTGAGCAAGAACTACCACCTGGAGAACGAAGTTGCGCGCC
TGAAGAAGCTGGTGGGTGAACTGCAGATGACGTCATGCGCGCGGATCCGAATTCTCCGGATCTG
GCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAA
TGGGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCG
CTACACTTGCCAGCGCCCTATCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTT
CGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTA
CGGCACCTCGACCCCAAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGAT
AGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAAC
TGGAACAACACTCAACCCTATAAGCTTAAGCTGGTCATATGACAATTCGCGCGCTGACCTCGGA
AATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGA
GACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTT
CCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTCTCGAGTTGCAGCTTTT

Vx(458)414
AATGTGTGCCTGGCGATCTCGAGGAGGATAAGGATCGATGGGGATCCGATCCAGCTGCTCTGAA
GCGAGCTCGGAACACTGAAGCTGCTCGACGGAGCCGAGCTCGGAAGCTGCAACGAATGAAGCAG
CTGGAAGACAAGGTGGAGGAACTGCTGAGCAAGAACTACCACCTGGAGAACGAAGTTGCGCGCC

TGAAGAAGCTGGTGGGTGAACTGCAGATGACGTCATGCGCGCGGATCCGAATTCTCCGGATCTG
GCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAA
TGGGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCG
CTACACTTGCCAGCGCCCTATCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTT
CGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTA
CGGCACCTCGACCCCAAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGAT
AGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAAC
TGGAACAACACTCAACCCTATAAGCTTAAGCTGAATGTCATATGACTCGCGCGCTGACCTCGGA
AATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGA
GACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTT
CCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTCTCGAGTTGCAGCTTTT

Primers used for PCR:

XhoI sites (underlined) were introduced with a mismatched sequence on the primers.

Vx(448)212 forward primer
5′-AATGTGTGCCTGGCGATCTCGAGCAGCTGGAAGACAAGGTGGAGGAAC-3′

Vx(448)212 reverse primer
5′-AAAAGCTGCAACTCGAGGGGTTATTGTCTCATGAGCG-3′

Vx(XXX)414 forward primer
5′-AATGTGTGCCTGGCGATCTCGAGGAGGATAAGGATCGATGGGGATCCGATCC-3′

Vx(XXX)414 reverse primer
5′-AAAAGCTGCAACTCGAGAAACAGGAAGGCAAAATGCCGC-3′