Supplementary Materials for Haeusler et al., "FRET Studies of a Landscape of Lac Repressor Mediated DNA Loops," *Nucleic Acids Research*.

LacI Expression and Purification

Protein expression and purification was adapted from published work (1,2), unpublished changes by Laurence Edelman, and additional modifications. LacI was expressed in *E. coli* BL21(DE3) pLysS previously transformed with pETLE1a, a plasmid that contains the LacI gene and ampicillin resistance. Cells were grown from a single colony in a 1 mL LB starter culture overnight, then added to 1 L of LB media containing 50 μg/mL of both ampicillin and chloramphenicol for a 2 hr growth at 37 °C. The cells were then induced with 0.1 mM IPTG and an additional 50 μg/mL of ampicillin. Growth was continued for 3 hrs, after which cells were harvested by pelleting at 8,000 *g* at 4 °C for 10 min in a JLA-10.5 rotor (Beckman, Avanti J-25I). The cell pellet was resuspended to 0.25 g/mL in LacI Resuspension Buffer (200 mM Tris-HCl, 200 mM KCl, 10 mM $MgCl_2$, 5% (v/v) glycerol, 0.3 mM DTT, 1 mM NaN_3, 1 mM PMSF, pH 7.2) and lysed by four passes through a French Press (American Instrument Company). The lysate was mixed with 0.4 mg/mL of DNase I for 30 min on ice with constant rocking. The debris was pelleted from the lysate at 39,000 *g* at 4 °C for 20 min using a JA 25.5 rotor. The crude LacI was precipitated from the supernatant with ~35% ammonium sulfate saturation (confirmed by a 1% SDS-PAGE) at 4 °C, and then spun again for 20 min at 39,000 *g*. The LacI pellet was resuspended in 20 mL of dialysis buffer (50 mM K_2HPO_4/KH_2PO_4, 0.1 mM EDTA, 5% (w/v) glucose, 0.3 mM DTT, 1 mM NaN_3, pH 7.2) and dialyzed versus the same buffer overnight at 4 °C using a Slide-A-Lyzer 7K cassette. The dialyzed sample was spun at 39,000 *g* for 20 min at 4 °C and the supernatant filtered through a 0.2 μm syringe filter (Millipore).

LacI was purified on a Mono S HR 5/5 Column using a GE AKTA FPLC with a 50 mM to 500 mM K_2HPO_4/KH_2PO_4 (0.1 mM EDTA, 5% (w/v) glucose, 0.3 mM DTT, 1 mM NaN_3, pH 7.2), 40-column volume linear gradient and a 1.0 mL/min flow rate. The fractions containing LacI (usually eluting between 250 and 300 mM KCl) were pooled and concentrated (if required) with a Centricon (Amicon, Millipore). Fractions that contain <95% purity (determined from a

1% SDS-PAGE silver staining analysis) were further purified using a Sephadex 300, 2 x 15 cm gel filtration column at 1.0 mL/min flow rate using LacI Storage Buffer (200 mM KPO_4, 2 mM EDTA, 400 mM NaCl, 1 mM DTT, 1 mM NaN_3, pH 7.2). The concentrated samples were dialyzed against LacI Storage Buffer G (LacI Storage buffer that includes 50% glycerol) with multiple changes at 4 °C overnight.

The concentration of LacI tetramer was determined from the A_{280} with the extinction coefficient of 22,500 for each monomer in the tetramer with a typical yield of 5 nmol (2 mL of 25 μM) LacI tetramer per liter of LB. The active protein concentration was determined by EMSA, using 7.5% native acrylamide gels (75:1 acrylamide:*bis*-acrylamide) run at 20 V/cm for 2 hours at 20 °C in TBE buffer. The radiolabeled DNA (14.4 nM) was incubated with [LacI] ranging from 0-32 nM in LacI buffer (25 mM Tris, 100 mM KCl, 5mM $MgCl_2$, 2 mM DTT, 50 μg/mL BSA, 0.02 % Nonidet P40 (now IGEPAL) detergent, pH 7.8) for 15 min at room temperature before loading into the gel. The fraction of DNA shifting was used to calculate the active LacI concentration relative to the A_{280}. Radiolabeled constructs were prepared by labeling 100 μM of the D_E Top (unmodified) primer with 70 μCi γ-^{32}P-ATP (Perkin-Elmer), following the manufacturer's protocol for T4 polynucleotide kinase (NEB). The primer was separated from radioactive ATP using a P6 column (BioRad). The labeled primer was mixed with the A_E Bot (unmodified) primer and a radiolabeled 9C14 construct was synthesized following the PCR protocol for externally positioned fluorophores described in Materials and Methods. The labeled product was visualized on a STORM imager and gel purified. The final DNA concentration was determined by scintillation counting (Packard 1600TR) with the specific activity being measured relative to the labeled primer.

Estimation of DNA Labeling Efficiency and Donor Quantum Yield Uncertainties

Based on the manufacturer's extinction coefficients for the dyes and for each primer, the labeling efficiencies for acceptor (f_A) and donor (f_D) are each ~100 % for labeling of the primers. However, given the low yields from PCR, then subsequent gel purification, storage, and use of

the doubly-labeled dsDNA, it is difficult to quantify the labeling efficiencies precisely. Due to all of the uncertainties in the labeling efficiencies and the quantum yields of conjugated dyes in the experimental setting, we have not corrected the apparent energy transfer efficiency or calculated inter-fluorophore distances from apparent FRET efficiency. However, since each FPV shared one fluorophore with another FPV, we could measure the differences in the relative labeling efficiencies of D_E vs. D_I and A_E vs. A_I using the emission spectra for direct excitation of each fluorophore. These measurements were performed on the four FPVs of 9C14 as representative molecules for the four FPV landscapes and applied to the entire landscape, as described in the Materials and Methods. The comparison of relative intensities of D_E vs. D_I and A_E vs. A_I showed that D_E had 70 % the labeling efficiency of D_I and A_I had 76 % the labeling efficiency of A_E. Including this correction in the analysis does not change the FRET results significantly and has no effects on our interpretation of loop population distributions. All of the landscape constructs were prepared in parallel using the same labeled primer stocks, and FRET was measured for the entire landscape on the same day, so we believe comparisons of FRET efficiencies within and among the FPV landscapes are robust.

FRET was calculated for donor enhancement and acceptor quenching, and these values are available in the Supplementary Materials in the Microsoft Excel file energy_transfer.xls. Only acceptor enhancement is reported in work reported in the text, because while changes to donor quantum yield due to LacI and IPTG binding were clearly present, they could not be directly measured for each individual construct. It is apparent in our singly labeled donor-only samples for 9C14 that the addition of LacI has dramatic effects on the quantum yield (Figure S1). We could not distinguish between donor quantum yield changes or energy transfer to an acceptor molecule for a doubly labeled sample in the presence of LacI, LacI + IPTG, or LacI + IPTG + additional LacI. The quenching or enhancement with the addition of LacI observed for each donor position variant was generally consistent on qualitative evaluation of all similar donor position variants. However, a quantitative analysis of donor quenching/enhancement would require singly labeled controls for each FPV and each DNA construct.

Because the acceptor emission for each doubly labeled sample under each experimental condition could be directly measured, we are more confident in these calculated FRET values.

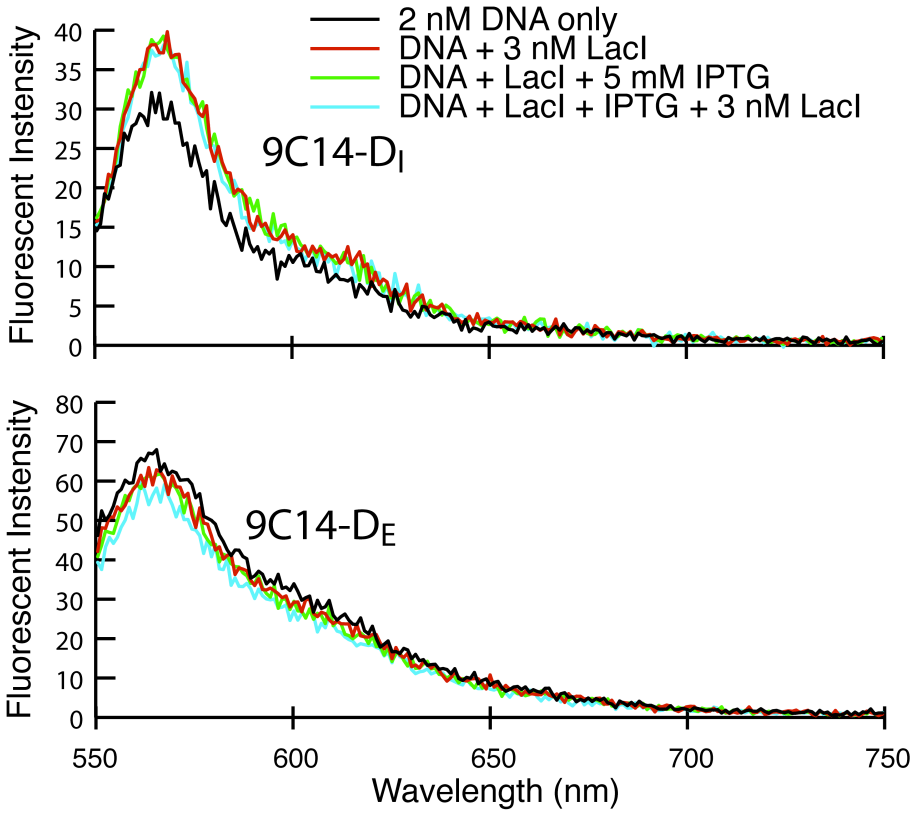

Figure S1. Donor quantum yield effects observed with the addition of LacI and/or IPTG. Singly labeled 9C14 donor only (D_I and D_E) samples were analyzed as described in the Materials and Methods. The addition of LacI had dramatic effects on the quantum yield of the donor in both cases. Presumably these changes in donor yield occur in all FPVs and are most likely caused by changes in the donor environment specific to each LacI•DNA complex. However, to quantitate the affects of donor quantum yield for the environment specific for each individual FPV requires two landscapes of controls for the D_I and D_E samples, which would be a total of 50 control samples. Therefore, as discussed in the Materials and Methods the acceptor, whose changes in quantum yield could be directly monitored throughout the experiments, provided a more reliable measurement of FRET seen as acceptor enhancement.

DNA Competition Experiments Demonstrate Stable Low-FRET LacI•DNA Loops

Competition experiments show that upon challenging loops with excess competitor DNA, after an initial small decrease FRET remains constant for at least 18 hours. The initial decrease is not accompanied by an increase in any other landscape, so we ascribe it to defective DNA or

proteins that form unstable loops as opposed to redistribution among loops. Pre-incubation with competitor gives no FRET, and addition of IPTG causes a rapid decrease in FRET, showing that the observed persistent FRET is due to kinetic stability of the loops, not instrumental artifacts.

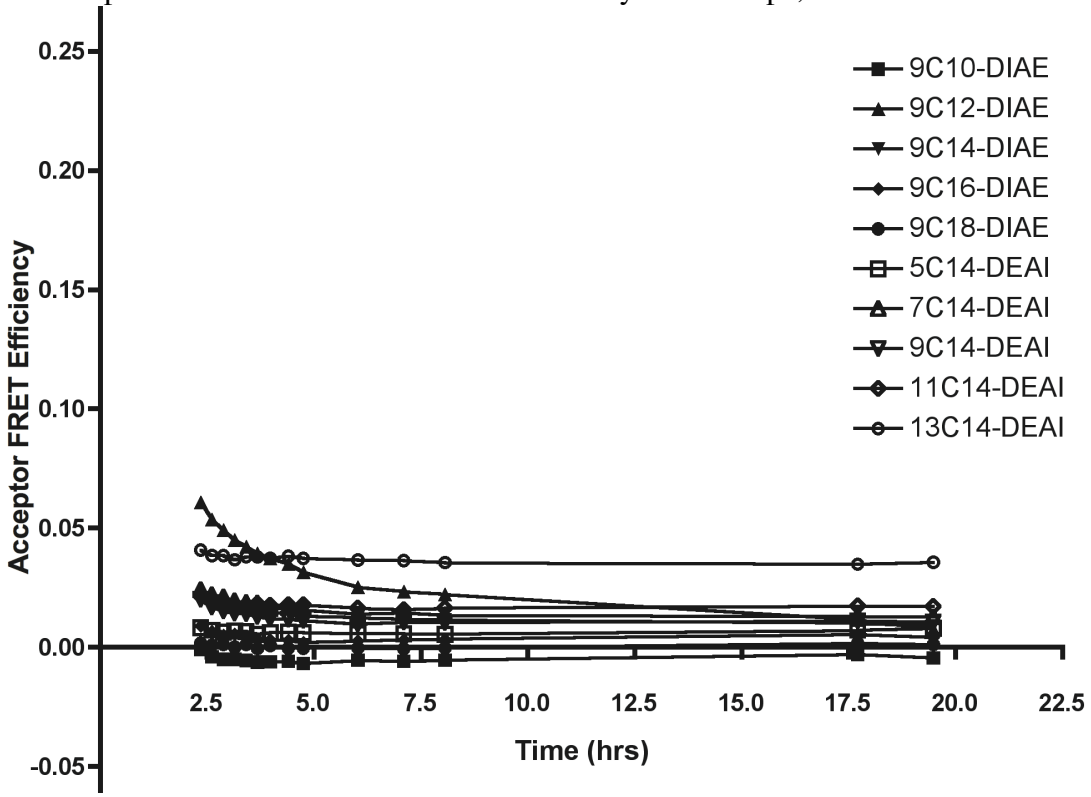

Figure S2. LacI•DNA loops were challenged with excess DNA competitor. The EMSA in Figure 4 of the main text exemplified, using the low-FRET construct 13C10, that low FRET constructs can form stable loops that are resistant to excess DNA competitor. Here we show FRET data for a selection of the landscape for the D_IA_E and D_EA_I FPVs that were competed with excess unlabeled DNA (10 nM unlabeled DNA construct, for example 9C14-D_IA_E was competed with unlabeled 9C14, and 9C18- D_IA_E was competed with unlabeled 9C18). following a two hour incubation of 2 nM DNA with 3 nM LacI. Acceptor FRET was measured on a Typhoon Imager (3) by exciting the donor (555 nm) and measuring acceptor enhancement (670 nm) as a function of time. FRET was calculated as shown in the Materials and Methods and then analyzed in Prism 4. The low-FRET constructs appear resistant to competitor challenge, suggesting that they are still forming stable loops with long half-lives, as seen with the EMSA studies.

Details of DNA Design

To facilitate modeling efforts or to allow for adapting this sequence landscape to other systems, we provide more details of the construction and all DNA sequences in Figure S3 and Table S1 below.

Figure S3. Graphical representation of the design for the DNA sequence landscape. The adaptor regions positioned between the central bend and the operators are highlighted for each of the construct combinations shown, green for the donor side and orange for the acceptor side. This set of molecules is the diagonal of the sequence matrix shown in the text figures. The two base pairs in each adaptor sequence that differ from one construct to the next are shown in lower case. In designing the sequences, pseudo LacI binding sites were disrupted. Unique restriction sites were added or removed in each adaptor region for convenience in cloning or restriction analysis of PCR products. The locations of the fluorophores are underlined and shown in bold, colored blue for donor and red for acceptor. The curved phased A-tract sequence is in italics, and the changing position of the arc illustrates the change in phasing relative to the operators. The four PCR primers used to generate the landscape are illustrated; sequences are in the text. All of the DNA sequences for the entire landscape are included in the table below.

TABLE S1. DNA Construct Sequence Landscape

Construct Label	DNA Sequence (between EcoR V sites)	Center-Center Operator Distance (bp)
5C10	ATCTGCAGGTCAGTCTAGGTAATTGTGAGCGCTCACAATTAGATCTTCGTACGGAT CCGGTTTTTTGCCCGTTTTTTGCCGTTTTTTGCCCGTTTTTTGCCGTTTTTTGCCC GTTTTTTGCCGTTTTTTGCCCGTTTTTTGCGCTGAACGCGTCCATCGAAGCTAGCT AATTGTGAGCGCTCACAATTCGTTGTGGTAAAGCTTTGAT	148
5C12	ATCTGCAGGTCAGTCTAGGTAATTGTGAGCGCTCACAATTAGATCTTCGTACGGAT CCGGTTTTTTGCCCGTTTTTTGCCGTTTTTTGCCCGTTTTTTGCCGTTTTTTGCCC GTTTTTTGCCGTTTTTTGCCCGTTTTTTGCGCTGAACGCGTCCTAATCGAAGCTAG CTAATTGTGAGCGCTCACAATTCGTTGTGGTAAAGCTTTGAT	150
5C14	ATCTGCAGGTCAGTCTAGGTAATTGTGAGCGCTCACAATTAGATCTTCGTACGGAT CCGGTTTTTTGCCCGTTTTTTGCCGTTTTTTGCCCGTTTTTTGCCGTTTTTTGCCC GTTTTTTGCCGTTTTTTGCCCGTTTTTTGCGCTGAACGCGTCCTAGAATCGAAGCT AGCTAATTGTGAGCGCTCACAATTCGTTGTGGTAAAGCTTTGAT	152
5C16	ATCTGCAGGTCAGTCTAGGTAATTGTGAGCGCTCACAATTAGATCTTCGTACGGAT CCGGTTTTTTGCCCGTTTTTTGCCGTTTTTTGCCCGTTTTTTGCCGTTTTTTGCCC GTTTTTTGCCGTTTTTTGCCCGTTTTTTGCGCTGAACGCGTCCTAGACGATCGAAG CTAGCTAATTGTGAGCGCTCACAATTCGTTGTGGTAAAGCTTTGAT	154
5C18	ATCTGCAGGTCAGTCTAGGTAATTGTGAGCGCTCACAATTAGATCTTCGTACGGAT CCGGTTTTTTGCCCGTTTTTTGCCGTTTTTTGCCCGTTTTTTGCCGTTTTTTGCCC GTTTTTTGCCGTTTTTTGCCCGTTTTTTGCGCTGAACGCGTCCTAGACGCTATCGA AGCTAGCTAATTGTGAGCGCTCACAATTCGTTGTGGTAAAGCTTTGAT	156
7C10	ATCTGCAGGTCAGTCTAGGTAATTGTGAGCGCTCACAATTAGATCTCTTCGTACGG ATCCGGTTTTTTGCCCGTTTTTTGCCGTTTTTTGCCCGTTTTTTGCCGTTTTTTGC CCGTTTTTTGCCGTTTTTTGCCCGTTTTTTGCGCTGAACGCGTCCATCGAAGCTAG CTAATTGTGAGCGCTCACAATTCGTTGTGGTAAAGCTTTGAT	150
7C12	ATCTGCAGGTCAGTCTAGGTAATTGTGAGCGCTCACAATTAGATCTCTTCGTACGG ATCCGGTTTTTTGCCCGTTTTTTGCCGTTTTTTGCCCGTTTTTTGCCGTTTTTTGC CCGTTTTTTGCCGTTTTTTGCCCGTTTTTTGCGCTGAACGCGTCCTAATCGAAGCT AGCTAATTGTGAGCGCTCACAATTCGTTGTGGTAAAGCTTTGAT	152
7C14	ATCTGCAGGTCAGTCTAGGTAATTGTGAGCGCTCACAATTAGATCTCTTCGTACGG ATCCGGTTTTTTGCCCGTTTTTTGCCGTTTTTTGCCCGTTTTTTGCCGTTTTTTGC CCGTTTTTTGCCGTTTTTTGCCCGTTTTTTGCGCTGAACGCGTCCTAGAATCGAAG CTAGCTAATTGTGAGCGCTCACAATTCGTTGTGGTAAAGCTTTGAT	154
7C16	ATCTGCAGGTCAGTCTAGGTAATTGTGAGCGCTCACAATTAGATCTCTTCGTACGG ATCCGGTTTTTTGCCCGTTTTTTGCCGTTTTTTGCCCGTTTTTTGCCGTTTTTTGC CCGTTTTTTGCCGTTTTTTGCCCGTTTTTTGCGCTGAACGCGTCCTAGACGATCGA AGCTAGCTAATTGTGAGCGCTCACAATTCGTTGTGGTAAAGCTTTGAT	156
7C18	ATCTGCAGGTCAGTCTAGGTAATTGTGAGCGCTCACAATTAGATCTCTTCGTACGG ATCCGGTTTTTTGCCCGTTTTTTGCCGTTTTTTGCCCGTTTTTTGCCGTTTTTTGC CCGTTTTTTGCCGTTTTTTGCCCGTTTTTTGCGCTGAACGCGTCCTAGACGCTATC GAAGCTAGCTAATTGTGAGCGCTCACAATTCGTTGTGGTAAAGCTTTGAT	158
9C10	ATCTGCAGGTCAGTCTAGGTAATTGTGAGCGCTCACAATTAGATCTCAATTCGTAC GGATCCGGTTTTTTGCCCGTTTTTTGCCGTTTTTTGCCCGTTTTTTGCCGTTTTTT GCCCGTTTTTTGCCGTTTTTTGCCCGTTTTTTGCGCTGAACGCGTCCATCGAAGCT AGCTAATTGTGAGCGCTCACAATTCGTTGTGGTAAAGCTTTGAT	152
9C12	ATCTGCAGGTCAGTCTAGGTAATTGTGAGCGCTCACAATTAGATCTCAATTCGTAC GGATCCGGTTTTTTGCCCGTTTTTTGCCGTTTTTTGCCCGTTTTTTGCCGTTTTTT GCCCGTTTTTTGCCGTTTTTTGCCCGTTTTTTGCGCTGAACGCGTCCTAATCGAAG CTAGCTAATTGTGAGCGCTCACAATTCGTTGTGGTAAAGCTTTGAT	154
9C14	ATCTGCAGGTCAGTCTAGGTAATTGTGAGCGCTCACAATTAGATCTCAATTCGTAC GGATCCGGTTTTTTGCCCGTTTTTTGCCGTTTTTTGCCCGTTTTTTGCCGTTTTTT GCCCGTTTTTTGCCGTTTTTTGCCCGTTTTTTGCGCTGAACGCGTCCTAGAATCGA AGCTAGCTAATTGTGAGCGCTCACAATTCGTTGTGGTAAAGCTTTGAT	156

TABLE S1. DNA Construct Sequence Landscape (continued)

Construct Label	DNA Sequence (between EcoR V sites)	Center-Center Operator Distance (bp)
9C16	ATCTGCAGGTCAGTCTAGGTAATTGTGAGCGCTCACAATTAGATCTCAATTCGTAC GGATCCGGTTTTTTGCCCGTTTTTTGCCGTTTTTTGCCCGTTTTTTGCCGTTTTTT GCCCGTTTTTTGCCGTTTTTTGCCCGTTTTTTGCGCTGAACGCGTCCTAGACGATC GAAGCTAGCTAATTGTGAGCGCTCACAATTCGTTGTGGTAAAGCTTTGAT	158
9C18	ATCTGCAGGTCAGTCTAGGTAATTGTGAGCGCTCACAATTAGATCTCAATTCGTAC GGATCCGGTTTTTTGCCCGTTTTTTGCCGTTTTTTGCCCGTTTTTTGCCGTTTTTT GCCCGTTTTTTGCCGTTTTTTGCCCGTTTTTTGCGCTGAACGCGTCCTAGACGCTA TCGAAGCTAGCTAATTGTGAGCGCTCACAATTCGTTGTGGTAAAGCTTTGAT	160
11C10	ATCTGCAGGTCAGTCTAGGTAATTGTGAGCGCTCACAATTAGATCTCAGATCTCGT ACGGATCCGGTTTTTTGCCCGTTTTTTGCCGTTTTTTGCCCGTTTTTTGCCGTTTT TTGCCCGTTTTTTGCCGTTTTTTGCCCGTTTTTTGCGCTGAACGCGTCCATCGAAG CTAGCTAATTGTGAGCGCTCACAATTCGTTGTGGTAAAGCTTTGAT	154
11C12	ATCTGCAGGTCAGTCTAGGTAATTGTGAGCGCTCACAATTAGATCTCAGATCTCGT ACGGATCCGGTTTTTTGCCCGTTTTTTGCCGTTTTTTGCCCGTTTTTTGCCGTTTT TTGCCCGTTTTTTGCCGTTTTTTGCCCGTTTTTTGCGCTGAACGCGTCCTAATCGA AGCTAGCTAATTGTGAGCGCTCACAATTCGTTGTGGTAAAGCTTTGAT	156
11C14	ATCTGCAGGTCAGTCTAGGTAATTGTGAGCGCTCACAATTAGATCTCAGATCTCGT ACGGATCCGGTTTTTTGCCCGTTTTTTGCCGTTTTTTGCCCGTTTTTTGCCGTTTT TTGCCCGTTTTTTGCCGTTTTTTGCCCGTTTTTTGCGCTGAACGCGTCCTAGAATC GAAGCTAGCTAATTGTGAGCGCTCACAATTCGTTGTGGTAAAGCTTTGAT	158
11C16	ATCTGCAGGTCAGTCTAGGTAATTGTGAGCGCTCACAATTAGATCTCAGATCTCGT ACGGATCCGGTTTTTTGCCCGTTTTTTGCCGTTTTTTGCCCGTTTTTTGCCGTTTT TTGCCCGTTTTTTGCCGTTTTTTGCCCGTTTTTTGCGCTGAACGCGTCCTAGACGA TCGAAGCTAGCTAATTGTGAGCGCTCACAATTCGTTGTGGTAAAGCTTTGAT	160
11C18	ATCTGCAGGTCAGTCTAGGTAATTGTGAGCGCTCACAATTAGATCTCAGATCTCGT ACGGATCCGGTTTTTTGCCCGTTTTTTGCCGTTTTTTGCCCGTTTTTTGCCGTTTT TTGCCCGTTTTTTGCCGTTTTTTGCCCGTTTTTTGCGCTGAACGCGTCCTAGACGC TATCGAAGCTAGCTAATTGTGAGCGCTCACAATTCGTTGTGGTAAAGCTTTGAT	162
13C10	ATCTGCAGGTCAGTCTAGGTAATTGTGAGCGCTCACAATTAGATCTCAGATCTCGT CGACGGATCCGGTTTTTTGCCCGTTTTTTGCCGTTTTTTGCCCGTTTTTTGCCGTT TTTTGCCCGTTTTTTGCCGTTTTTTGCCCGTTTTTTGCGCTGAACGCGTCCATCGA AGCTAGCTAATTGTGAGCGCTCACAATTCGTTGTGGTAAAGCTTTGAT	156
13C12	ATCTGCAGGTCAGTCTAGGTAATTGTGAGCGCTCACAATTAGATCTCAGATCTCGT CGACGGATCCGGTTTTTTGCCCGTTTTTTGCCGTTTTTTGCCCGTTTTTTGCCGTT TTTTGCCCGTTTTTTGCCGTTTTTTGCCCGTTTTTTGCGCTGAACGCGTCCTAATC GAAGCTAGCTAATTGTGAGCGCTCACAATTCGTTGTGGTAAAGCTTTGAT	158
13C14	ATCTGCAGGTCAGTCTAGGTAATTGTGAGCGCTCACAATTAGATCTCAGATCTCGT CGACGGATCCGGTTTTTTGCCCGTTTTTTGCCGTTTTTTGCCCGTTTTTTGCCGTT TTTTGCCCGTTTTTTGCCGTTTTTTGCCCGTTTTTTGCGCTGAACGCGTCCTAGAA TCGAAGCTAGCTAATTGTGAGCGCTCACAATTCGTTGTGGTAAAGCTTTGAT	160
13C16	ATCTGCAGGTCAGTCTAGGTAATTGTGAGCGCTCACAATTAGATCTCAGATCTCGT CGACGGATCCGGTTTTTTGCCCGTTTTTTGCCGTTTTTTGCCCGTTTTTTGCCGTT TTTTGCCCGTTTTTTGCCGTTTTTTGCCCGTTTTTTGCGCTGAACGCGTCCTAGAC GATCGAAGCTAGCTAATTGTGAGCGCTCACAATTCGTTGTGGTAAAGCTTTGAT	162
13C18	ATCTGCAGGTCAGTCTAGGTAATTGTGAGCGCTCACAATTAGATCTCAGATCTCGT CGACGGATCCGGTTTTTTGCCCGTTTTTTGCCGTTTTTTGCCCGTTTTTTGCCGTT TTTTGCCCGTTTTTTGCCGTTTTTTGCCCGTTTTTTGCGCTGAACGCGTCCTAGAC GCTATCGAAGCTAGCTAATTGTGAGCGCTCACAATTCGTTGTGGTAAAGCTTTGAT	164

References

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All of these references are also found in the reference list of the main text.