

Supplementary Information

Manipulating DNA writhe through varying DNA sequences

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EXTENDED EXPERIMENTAL PROCEDURES

Reagents: T4 DNA ligase, Nuclease BAL-31 and SacI were purchased from New England Biolabs (Ipswich, MA). Topo I was obtained from TopoGEN (Columbus, OH). Duplex linear DNA precursors were provided by Generay Biotech (Shanghai, China).

Preparations of circular DNA

A 50 μ l solution containing 50 mM Tris-HCl, 10 mM MgCl₂, 1 mM ATP, 10 mM dithiothreitol, 500 ng linear DNA and 20 U T4 DNA ligase was incubated at 16 °C for 8 hrs. The obtained circular DNA products were further analyzed using agarose electrophoresis (1.5%).

Reactions of topo I with Circular DNA 1: A solution containing 10 mM Tris-HCl, 150 mM NaCl, 0.1% BSA, 0.1 mM Spermidine, 5% glycerol, 200 ng Circle DNA 1 and 3 U topo I was incubated at 37 °C for 1 hr. The reaction products were further analyzed using agarose electrophoresis (1.5%).

Reactions of SacI with circular DNA: A solution containing 10 mM Bis-Tris-Propane-HCl, 10 mM MgCl₂, 1 mM Dithiothreitol, mixtures of linear DNA and circular DNA (200 ng) and 10 U SacI was incubated at 37 °C for 1 hr. The reaction products were further analyzed using agarose electrophoresis (1.5%).

AFM studies

Immobilization of DNA samples on micas were carried out following the previously reported procedures.^{S1,S2} AFM images were obtained in Tapping ModeTM on a MultimodeTM AFM (Veeco, Santa Barbara, CA) in connection with a Nanoscope VTM controller. Antimony (n) doped Si cantilevers with nominal spring constants between 20 and 80 N/m were selected. Scan frequency was 1.9 Hz per line and the modulation

amplitude was in a nanometer range. All DNA sample determinations were carried out in air at room temperature.

5' CGAAAAGTGCAAAAAGTCGGAAAAATCCGTGCAAAAATCGTCAAAAGGCCCGAAAAAATAGCTAAAAATCGTCGAAAAACTGCGTTGA
3' TCGAGCTTTTCACGTTTTTCAGCCTTTTATAGGCACGTTTTTAGCAGTTTTCCGGGCTTTTTATCGATTTTATAGCAGCTTTTGACGCAACT
AAAAGCTTAAAAACGATGCAAAAAGTGCATTCAAAAATGGGCAAAAAGTGGCCAAAAGCTATAAAAAACGCGCAAAAATCGCACTTTTGT
TTTCGAATTTTGTACGTTTTTCACGTAAGTTTTTACCCGTTTTTCACCGGTTTTTCGATATTTTTTGCGCGTTTTTAGCGTGAAAAAC
GAGTTTTTTCGGGCTTTTTTGGATCATTTTTTAGTCGTTTTTGGCCATTTTCGGCACTTTTTGCATATATTTGCCCGTTTTTGCCAATT
CTCAAAAAGCCGAAAAAACCTAGTAAAAATCAGCAAAAACCGGTAAAGCCGTGAAAAACGTATATAAAACGGGCAAAAACGGTTAA
TTTCGTATTTTCGCTATTTTTTGGCATTTTTTGGCCATTTTAGGCTTTTGGGTGGTTTTTCGGCCGTTTTTGGAGGTTTTCCAGATTTTGC
AAAGCATAAAAGCGATAAAAAACCGTAAAAAACCGGTAAAAATCCGAAAAACCCAAAAAGCCGGCAAAAACCTCCAAAAGGTCTAAAACG
ATTTTCAGTGCGTTTTTGGCCATTTTCGGCTGTTTTTGGCCATATATTTGCCCGTTTTTGCCAATTTTTTGCCAATTTTCGGGGTATTTT
TAAAAGTCACGCAAAAACCGGTAAAGCCGACAAAAACCGGTATATAAAACGGGCAAAAACCGTTAAAAAACGGTTAAAGCCCCATAAAA
CGCTATTTTTTGGCATTTTTTTGGCCATTTTGCAATGTTTTTTCAGTTTTTGTGCAAAAGCAGTGAAAAGTGGCCAAAATGCCGAAAAAT
GCGATAAAAAACCGTAAAAAACCGGTAAACGTTACAAAAAAGTCAAAAAACACGTTTTTCGTCACTTTTCACCGGTTTTTACGGCTTTTAA
CAGCGAAAAGCCGAAAAATGGCCGTAAAAATGGTAAAGAATTCAAAAATTCATAAAACCCAAAATGGCTGAGAAAAGGAGTGAAAAATG
GTCGCTTTTCGGGCTTTTACCGGCATTTTACCATTCTTAAGTTTTTAAGTGATTTTGGGTTTACCGACTCTTTTCCTCACTTTTAC
GTCCTAAAAACCGCAAAAATCTCTCAAAAATGAGCT 3' Linear DNA 1*
CAGGATTTTTGGCGTTTTTAGAGAGTTTTTAC 5'

- * (1) Junctions between the segments that are highlighted in red and in blue represent the points at which adenine-rich and thymine-rich segments alternate between two opposite strands; and
(2) This linear DNA contains two segments of continuous spaced adenine tracts in its sequence.

Fig. S1. Nucleotide sequences of Linear DNA 1 designed in our studies.

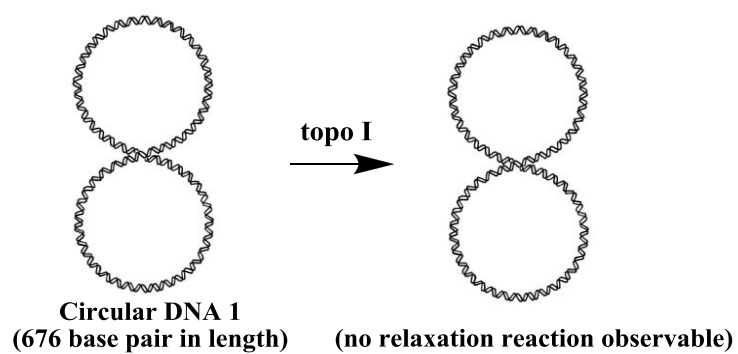


Fig. S2. Diagrammatic illustration of reaction of Circular DNA 1 with topo I.

A

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5' CGCTACATAATACGACTCACTATTATATGTATAACTTCGTATAATGTATGCTATACGAAGTTATTGCTCGCAGTGTTACTGCAATCAT
3' TCGAGCGATGTATTATGCTGAGTGATAATATACATATTGAAGCATATTACATACGATATGCTTCAATAACGAGCGTCACAATGACGTTAGTA
CGTGGTGATTAACTCTTGTGTGTAATTCGTTACTCAACGAAGGTTAATTCACTATAGTTGTCCTGGTACTCTCTAGTGAATTCCTTAAGTGA
GCACCACTAATTAGAACACACATTAAGCAATGAGTTGCTTCCAATTAAGTGATATCAACAGGACCATGAGAGATCACTTAAGGAATTCACT
GTAGTATTAAGAAGTAAGTGAAGATGCTTCGAGTTATGTGACTGATAAGTATTCAATCAAGTCATTCTGAGAATAATGTATGTTACTATAA
CATCATAATTTCTTCATTACATTTCTACGAAGCTCAATACACTGACTATTCATAAGTTAGTTCAGTAAGACTCTTATTACATACAATGATATT
TCATGATTAGAACTCGAGTTGCTCTTGCAATGGTGTCAACGTTGGATAAATACTGACATAGCAGAACTTTAAAAGTGTTTCATTATTGGAAGATC
AGTACTAATCTTGAGCTCAACGAGAACGTACCACAGTTGCAACCTATTATGACTGTATCGTCTTGAAATTTTCACAAGTAATAACCTTCTAG
TGCGAACATGCTCAACGTTCTTACCTCTGTTGAGATCCAGTTCGATATAATTCAGTTGTGCACCAACTTATCTTCAGCATTACTTTCACCA
ACGCTTGTACGAGTTGCAAGAATGGAGACAACCTTAGGTCAAGCTATATTAAGTGAACACGTGGGTGAATAGAAGTCGTAATGAAGTGGT
GAGTTTCTGTGTGAGTAATCGGGAACAGGAAGGCAAGATTCAAATCTTAAAGTGAATAAGTTCGACACAGAAATGTTGAATGCTCATATCTC
CTCAAAGACACACTCATTAGCCCTTGTCTTCCGTTCTAAGTTTGAATTTCACTTATCAAGCTGTGTCTTTACAACCTTACGAGTATGAG
TTCCTTTAGCGACTTCAATACGCTTATTGAAGCATTTATAAGGGTTATTGTTACGTGAGTATAACTTCGTATAATGTATGCTATACGAAGTT
AAGGAAATCGCTGAAGTTATGCGAATAACTTCGTAAATATTCCCAATAACAATGCACCTCATATTGAAGCATATTACATACGATATGCTTCAA
ATGGCTCGAAGTCAGTTATAGATATCTAAGTGAGCT 3' Linear DNA 2*
TACCGAGCTTCAGTCAATATCTATAGATTAC 5'

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* This linear DNA contains no spaced adenine tract in its sequence.

B

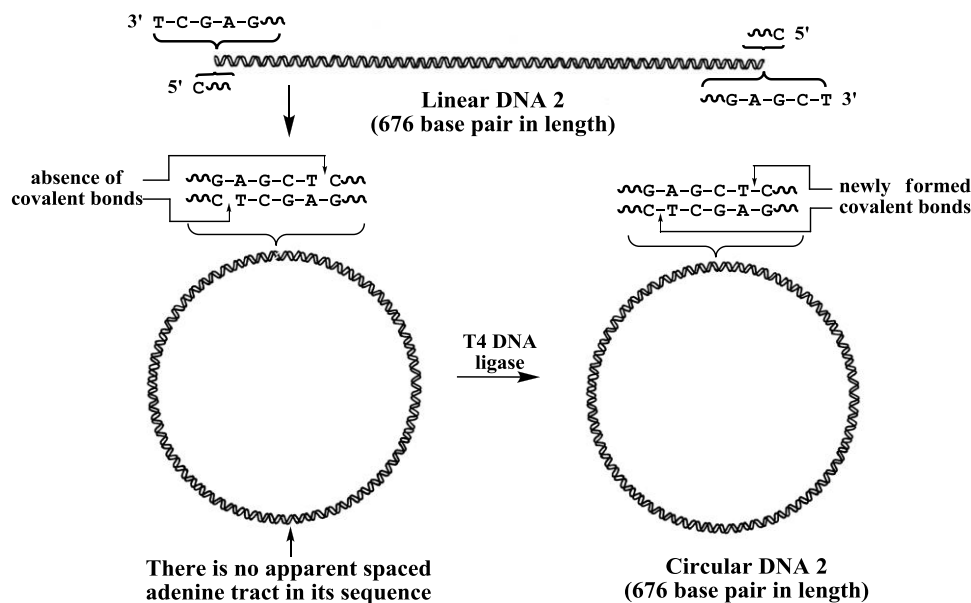


Fig. S3. Synthesis and confirmation of Circular DNA 2 (676 bp in length) from Linear DNA 2 (676 bp in length). (A) Nucleotide sequences of Linear DNA 2; (B) Diagrammatic illustration of synthesis of Circular DNA 2 from Linear DNA 2

3' 5' CAGTTGGGTAATTTTATAGGGTTTTCCAGTTTTGACGTTGTTTTTCGACGGAATTCCTTTTACGACTCACTTTTGCCTTGACTAG
TCGAGTCAACCCATTAAAAATCCCAAAAGGGTCAAAACTGCAACAAAAAGCTGCCTTAAGGGAAAAATGCTGAGTGAAAAACGGAACGATC
AGGGTTTTTACCAAGCTTTCTATTTTGGTCTTTTGCCATAACTTTTTATAGCATACATTTTACGAGTTTTATAAGCTGTTTTTCATGAGGC
TCCCAAAAATGGTTCGAAAGATAAAAAACGAGAAACGGTATTGAAAAATATCGTATGTAAATGCTCAAAATATTCGACAAAAAGTACTCCG
TTCTTTTATAGGTTTTTGTCTATGTTTTAATGGTATCTTTTTCGTCGGTGGCATTTTTCGGGGTTTTGCGCGGATCCCTTTTGTATG
AAGAAAAATATCCAAAAACAGTACTAAATTACCATAGAAAAAGCAGCCACCGTAAAAAGCCCCAAAAACGCGCTAGGGGAAAAACAAATAC
GGCCTTTTTACATCAGGTTTTTTCGGCTCAGCAATGATTTTTGCCCCTTTTAGATTTTTCAATGATATTTTAGGCGTTTTTGACGTTTTTCAG
CCGGA AAAATGTAGTCCAAAAAGGCGAGTCTTACTAAAAACGGGAAAAATCTAAAAAGTTACTATAAAAAATCCGCAAAAAACGCAAAAGTC
TTTTTCGGTGTGCGCCTTTTTTCCCTTTTTTGGCATTTTTTTCGGCATTTTTTTGCATATATTTTTTGGAGTTGTTTTGATCCGTTTTGATTT
AAAAAGGCACAGCGGAAAAAGGGA AAAACGCGTAAAAAGCCGTAAAAAGCGTATATAAAAAACCTCAACAAAACTAGGC AAAAATAA
TCAGTGCCTTTTTGGCCATTTTGCCCTAGTTTTTTGCGTTGCTATTTTTTGTTAATTTTTGCCAATTTTCGTATTTTCGCTATTTTTTGGCAT
AGTCACGCAAAAACCGGTAAAAACGGATCAAAAAACGCAACGATAAAAAACAATTAAAAACGGTTAAAAAGCATAAAAAGCGATAAAAAACCGTA
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AAAAACTGGTAAAAAGAACAAAACCTACCAAAAAGCCGGCAAAAAACCTCAACTTAAATGCAGGTCTAAACTAAAAAGTCACGCAAAAACCGG
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TAAACGCTCACAAAAACGGAGACGATAAAAAACAATTAAAAACGGTTAAAAAGCTCCATAAAAGCGATAAAAAACCGTAAAAAACAGAGTAA
TTTTAGTCGTTTTTGGCCATTTTTCCCTGTTTTTGCTCACCCTTTTTCGCTGGTGCCGAGTTTTTGATGCTTTTTTGACGTTTTTGTGACGAGT
AAAACTCAGCAAAAACCGGTAAAAAGGACAAAAACGAGTGGGTAAAAAGCGACCGGCTCAAAAACTACGAAAAACGTCAAAACACGTGCTCA
TTTTGACATCGGACTGGTTTTTCACAGCGGTTTTTCAGGCTTTTTGCACAACATTTTTCATGTATTTTGAAGGAGAGAAGATTTTGGGCTCAGT
AAAACGTAGCTGACCAAAAGTGTGCGCAAAAGTCCGAAAAACGTGTTGTAAAAAGTACATAAACTTCTCTCTCTTAAAAACCCGAGTCA
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AAACTATGGGCTGCTAAAACTGTGGTGCTAAAAACGTCCGCAAAAACTCGGTAAAAACGCATATCGTAAAAACCTATGCTACAAAAGGTACAA
TTAGTGGTTTTTGACGTAGCTTTTCGAAGCTTTTAGTCATTTTTATAGCTGTTTTTGTGTGAGATTTTATCCGCTCACTTTTCGAATTCCTT
AATCACC AAAAAGCTTCGAAATCAGTAAAAATATCGACAAAAACACACTCTAAAAATAGGCGAGTGAAGGCTTAAGGAA
TTTACGAGCCGATTTTTCGGGTGTGGCTTTTTGTCCGTTTTTCTGTTTGAAGT 3' Linear DNA 3*
AAATGCTCGGCCTAAAAACGCCACACCGAAAAACAGGCAAAAGGACAAAC 5'

- * (1) Continuous spaced adenine tracts occur exclusively in one of the double strands of this linear DNA; and
(2) The duplex segments highlighted in blue and green are those that possess high and low degrees of curvatures separately.

Fig. S4. Nucleotide sequences of Linear DNA 3.

5' CGAAAAGTCGAAAAGTCGGAAGATCCGTGCAAAATCGTCAAAAGAATTCAAAAATAGCTAAAAATCGTCGAAAACGCGTTGA
3' TCGAGCTTTTACGTTTTTACGCTTTTATAGGCACGTTTTTAGCAGTTTTCTTAAGTTTTTATCGATTTTTAGCAGCTTTTACGCAACT
AAAAGCTTAAAAACGATGCAAAAAGTGCATTCAAAAATGGGCAAAAAGTGGCCAAAAGCTATAAAAAACGCGCAAAAATCGCAGTTTTTT
TTTTCGAATTTTTGCTACGTTTTTACGTAAGTTTTTACCGGTTTTTACCGGTTTTTCGATATTTTTTGCAGTTTTTACGCTGAAAAA
GCATATATTTTTTGGAGCTTTTGTATCCGTTTTGATTTTTAGTGCCTTTTTGGCCATTTTGCCCTAGTTTTTGCCTTGTATTTTTGT
CGTATATAAAAAACCTGCAACAAAACCTAGGCAAACTAAAAGTCACGCAAAAACCGGTAAAACGGGATCAAAAACGCAACGATAAAAAACA
TAATTTTTGCAATTTTTCGGTATTTTCGCTATTTTTTGGCATTTTTTGACCATTTTTCTTGTGTTTGGATGGTTTTGCGGAAAAAGTGGCGA
ATTAACCAAGGTTAAAGCCATAAAAGCGATAAAAAACCGTAAAAAACTGGTAAAAAGAACAAACCTACCAAAACGGCTTTTTACCGCT
AAAGTGCAAAAGTCGGAAGGAGTCAAAAGTGGCCAAAATGCCGAAAATCAGCGAAAAGGATTCAAAAATCACTAAACCCAAAAT
TTTACGTTTTTACGCTTTTCTGAGTTTTTACCGGTTTTTACGGCTTTTATAGTCGTTTTCTTAAGTTTTTAAGTATTTTGGGTTTTA
GGCTGAGAAAATGGGCAAAAAGTGGCCAAAAGCTATAAAAAATCCGTGCAAAAATCGTCAAAAGGCCGCAAAAATAGCTAAAAAGCAAT
CCGACTCTTTTACCGTTTTTACCGGTTTTTTCGATATTTTTTATAGGCACGTTTTTACGAGTTTTTCCGGGCTTTTTATCGATTTTTCGTTA
GAAAACTGCGTTGAAAAAGGTAAAAACGATGCAAAAAGTGCATTCAAAAACGCGCAAAAACCGCAAAAATCTCTCAAAAATGAGGTAAAA
CTTTTTCGCACTTTTTCGCTACGTTTTTACGTAAGTTTTTGCAGTTTTTGGCGTTTTTGGCGTTTTTACGAGTTTTTACCTCCATTTT
ATGGCCGTAAAAATGGTAAAGGAGTGAAGAGCCGAAAAATGGTCTAAAAATCGCACCGGCGTTTTTTGGAGTTGAATTTTACGTCAG
TACCGGCATTTTACCATTTTCTCACTTTTTCGGGCTTTTTTACCGGATTTTATAGCTGGCCGGCAAAAACCTCACTTAAAAATGCAGGTC
ATTTGATTTTTCAGTGCCTTTTGGCCATTTTGTCTTTTTTGGCTCTGCTATTTTTGTTAATTTTGGCAATTTTTCGAGGTATTTTCGCTA
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GAAAAGCCGAAAAATGGCGTAAAAATGGTAAAGGAGTAAAAATCACTAAACCCAAAATGGCTGAGAAAAGTAAAAATGAGTCC
CTTTTCGGGCTTTTTACCGGATTTTACCATTTTCTCACTTTTTAAGTATTTTGGGTTTTTACCGACTCTTTTCTTAAGTTTTTACAG
TAAAAACCGCAAAAATCTCTCAAAAATGAGCT 3' Linear DNA 5'
ATTTTGGCGTTTTTACGAGTTTTTAC 5'

- * (1) Junctions between the segments that are highlighted in red and in blue represent the points at which adenine-rich and thymine-rich segments alternate between two opposite strands; and
(2) This linear DNA contains four segments of continuous spaced adenine tracts in its sequence.

Fig. S6. Nucleotide sequences of Linear DNA 5.

A

5' CTAGATCATAGTCGCAATTAACAGATTAAGTTGAGTAACACCAGAGTTCACAGTCACGAAGTTGTAATTAACGACGACCAGTCAGTAA
3' TCGAGATCTAGTATCAGCGTTAATTGTCTAATTCAACTCATTTGGTCTCAAGTGTGAGTCTCAACATTAATTGGTCTGGTCAGTCATT
TACGACTCACTTAAGACATTGACTAGAGGATACCAACATAGGTATATAGAACCAATCTAGAGCCATAACTTCGTATAGAATACATTATACGA
ATGCTGAGTGAAATTCGTAACTGATCTCTATGGTTGTATCCATATATCTTGGTTAGATCTCGGTATTGAAGCATATCTTATGTAATATGCT
AGTTATATAAGATGTCAAACATGAGAATTATGTTATAGTTAAATGTAATGATAATAATGATTTCTTAGAAGTCAGATGACACTTTTCAGAG
TCAATATATTTACAGTTTGTACTCTTAATAACAATATCCAATTACATTACTATTATTACTAAAGAATCTTCAGTCTACTGTGAAAAGTCTC
AAATGTAAGCAGAACACATATTTATTTATTTCTAAATACATTCAAAATATGTATCAGCTCATGAGACAATAACCATGATAAATGATTCAATAA
TTTACATTCTGCTTGTGTATAAATAAATAAGATTTATGTAAGTTTATACATAGTCGAGTACTCTGTTATTGGTACTATTACTAAGTTATT
TATTGAATTAAGGAAGAGATGAGTATTTCAACATTTCCGTGTCTTATTTCCCTTATAAGCGACTTATTGCCTTCATGTTCCCTTTGATCAC
ATAACTTAATTTCTCTCATCTCATAAGTTGTAAGGCACAGTGAATAAGGGAATATTCGCTGAATAACGGAAGTACAAGGAAGTACTG
CCAGGAATTCGGTGAAGATGATGAAGATAAGTTGGGTGAACGAATGGATTACATAGAAGTGGATCTCAACAGAGGTAAGTTAAG
GGTCTTAAGACCACTTTCATTCTCTACTACTTCTATTCAACCCACTTGCTTACCTAATGTATCTTGACCTAGAGTTGTCCTCAATTC
ATTTGCACAACATGAAGGATCATGTAACTAGAATTGATAGAAGGAGAGAAGAGATGGAGCTCAATGAAGCCATACCAAAACGACGAGCGTGAC
TAAACGTGTTGTACTTCTTAGTACATTGATCTTAACATATCTTCTCTCTTCTACCTCGAGTTACTTCGGTATGGTTTGTCTGCTCGCACTG
ACAACGATGACTGCAGGAATTAATAGAGCCATAACTTAGTATAGCATACATTATACGAAGTTATCCATGGACTAGTGAGTCGTATTACGTAG
TGTGCTACTGACGTCCTTAATTATCTCGGTATTGAATCATATCGTATGTAATATGCTTCAATAGGTACCTGATCCTCAGCATAATGCATC
ATTTGAGTAATAATGGTCATAGCTGTTTACTGTATGAAATTTGTTATAAGCTCACAATTACACACAACATACGAGCCGGAAGCATAAAGTGA
TAACCTCATTATTACAGTATCGACAAATGACATACTTTAACAATATTCGAGTGTAAATGTGTGTGTATGCTCGGCCTTCGTATTTCACAT
AAGTGAGAGGAATTAACCATGGATCAGGTAAGTGATATCGAAGACTTAACGCTAGAATTTCGATAACCTATAGTGAGTCGTATTACATGGTCA
TTCACTCTCTTAATTTGGTACCTAGTCCATTCACTATAGCTTCTGAATTGCGATCTTAAGCTATTGGATATCACTCAGCATAATGTACAGT
TAGCTGTTCTGGCAGCTCTGACCAATGTCTCAATCAATCTATGATGTTACATTGCACAAGATAAAGGAATATATCATCATGAACAATAACCA
ATCGACAAGACCGTCGAGACTGGTTACAGAGTTAGTTAGATACTACAATGTAACGTGTTCTATTTCCTTATATAGTAGTACTTGTATTGGT
ACTGCTGATTACATAAACAGTAATACGAGCT 3' Linear DNA 6*
TGACAGACTAATGTATTGTCATTATGC 5'

* This linear DNA contains no spaced adenine tract in its sequence.

B

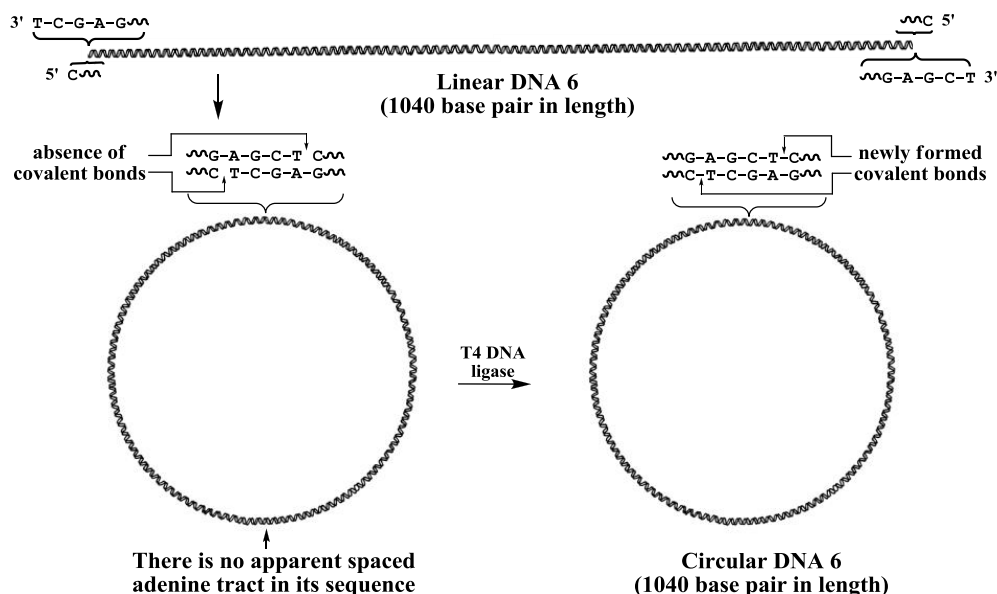


Fig. S7. Synthesis and confirmation of Circular DNA 6 (1040 bp in length) from Linear DNA 6 (1040 bp in length). (A) Nucleotide sequences of Linear DNA 6; (B) Diagrammatic illustration of synthesis of Circular DNA 6 from Linear DNA 6.

References for Supplementary Information:

- S1. Y. L. Lyubchenko and L. S. Shlyakhtenko, *Methods*, 2009, **47**, 206-213.
- S2. Y. L. Lyubchenko, A. A. Gall, L. S. Shlyakhtenko, R. E. Harrington, B. L. Jacobs, P. I. Oden and S. M. Lindsay, *J. Biomolec. Struct. Dyn.*, 1992, **9**, 589-606.