Electronic Supplementary Material (ESI) for Chemical Communications

Electronic Supplementary Information

DNA gyrase-driven generation of G-quadruplex from plasmid DNA

Bei Lv‡, Dawei Li‡*, Hao Zhang, Jasmine Yiqin Lee and Tianhu Li*

EXTENDED EXPERIMENTAL PROCEDURES

Detailed experimental descriptions of Fig. 1B. Electrophoretic analysis of intermediate DNA molecules generated during synthesis of Circular DNA 1. Lane M: molecular weight markers; Lane 1: Linear DNA 1 (Fig. 1A). This linear DNA was obtained through PRC amplification based on the standard procedures reported in literature[1] using a plasmid DNA of X2420 as the template (plasmid DNA X2420 was purchased from Generay Biotech in Shanghai, China) (Step 1 in Fig. 1A). The sequences of the forward and reverse primers were 5'CCGAGCTCAGGATCCGGATGATCCCTAACCCTAACCCTAACCCTAACCAGTCCGTA ATACGACTCAC 3' (ssODN-1) and 5' TCGTTTGGTATGGCTTCATT 3' (ssODN-2) respectively; Lane 2: Linear DNA 2 (Fig. 1A). This cohesive end-containing linear DNA was obtained through incubation of a solution that contained 10 mM Bis-Tris-propane-HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, Linear DNA 1 (500 ng) and 10 U SacI at 37 ℃ for 1 hr. (Step 2 in Fig. 1A); Lane 3: Crude Circular DNA 1 (Fig. 1A). This rude circular DNA (Circular DNA 1) was obtained through incubation of a solution (50 μl) that contained 50 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM ATP, 10 mM dithiothreitol, 500 ng Linear DNA 2 and 20 U T4 DNA ligase at 16 ℃ for 8 hrs (Step 3 in Fig. 1A); Lane 4: pure Circular DNA 1 (Fig. 1A). This pure circular DNA was obtained through incubation of crude Circular DNA 1 (the same batch of DNA sample as the one loaded in Lane 3) with 2 U exonuclease BAL-31 at 30 ℃ for 10 hrs.

Detailed experimental descriptions of Fig. 2B. Electrophoretic analysis of products of enzymatic reactions on Circular DNA 1. Lane M: molecular weight markers; Lane 1: Circular DNA 1 alone; Lane 2: non-G-quadruplex-containing negative supercoil of DNA (Structure 1 in Fig. 2A) formed from Circular DNA 1 by the action of DNA gyrase under a non-physiological concentration of potassium ion (24 mM KCl). This DNA was obtained through incubation of a

solution (50 μl) that contained 35 mM Tris-HCl, 24 mM KCl (a non-physiological concentration of potassium ion), 4 mM $MgCl_2$, 2 mM DTT, 1.75 mM ATP, 5 mM spermidine, 0.1 mg/ml BSA 6.5% Glycerol, 500 ng Circular DNA 1 (the same batch of DNA sample as the one loaded into Lane 1 in Fig. 2B) and 1 U DNA gyrase at 37 ℃ for 1 hr.; Lane 3: G-quadruplex-containing negative supercoil of DNA (Structure 2 in Fig. 2A) formed from Circular DNA 1 by the action of DNA gyrase under a physiological concentration of potassium ions (150 mM KCl). This DNA was obtained through incubation of a solution (50 μl) that contained 35 mM Tris-HCl, 150 mM KCl (a physiological concentration of potassium ions), 4 mM NaCl, 4 mM $MgCl_2$, 2 mM DTT, 1.75 mM ATP, 5 mM spermidine, 0.1 mg/ml BSA, 500 ng Circular DNA 1 (the same batch of DNA sample as the one loaded into Lane 1 in Fig. 2B) and 2 U DNA gyrase at 37 ℃ for 1 hr.; Lane 4: nicked form of G-quadruplex-containing circular DNA (Structure 3 in Fig. 2A). This nicked form of DNA was obtained through incubation of a solution that contained 20 mM Tris-acetate, 50 mM potassium acetate, 10 mM magnesium acetate, 1 mM Dithiothreitol, 200 ng of G-quadruplex-containing negative supercoil of circular DNA (the same batch of DNA sample as the one loaded into Lane 3 in Fig. 2B) and 10 U Nt.BsmAI at 37 ℃ for 1 hr.; Lane 5: G-quadruplex-containing closed circular DNA in its relaxed form (Circular DNA 2 in Fig. 2A). This G-quadruplex-containing circular DNA was obtained through incubation of a solution (50 μl) that contained 50 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM ATP, 10 mM dithiothreitol, 500 ng nicked form of G-quadruplex-containing circular DNA (the same batch of DNA sample as the one loaded into Lane 4 in Fig. 2B) and 20 U T4 DNA ligase at 16 ℃ for 8 hrs (Step 4 in Fig. 2A).

Detailed experimental descriptions of Fig. 3B. Electrophoretic analysis of DNA products generated upon the action of DNA gyrase and other enzymes on Circular DNA 1 under non-

physiological conditions [the studies shown in this section (non-physiological concentration of cations) serve as control experiments for the studies shown in Fig. 2A (physiological concentration of cations). Lane M: molecular weight markers; Lane 1: Circular DNA 1 alone; Lane 2: non-G-quadruplex-containing negative supercoil of DNA (Structure 1 in Fig. 3A) formed from Circular DNA 1 by the action of DNA gyrase under a non-physiological concentration of potassium ion (24 mM KCl). This DNA was obtained through incubation of a solution (50 μl) that contained 35 mM Tris-HCl, 24 mM KCl (a non-physiological concentration of potassium ion), 4 mM $MgCl_2$, 2 mM DTT, 1.75 mM ATP, 5 mM spermidine, 0.1 mg/ml BSA 6.5% Glycerol, 500 ng Circular DNA 1 (the same batch of Circular DNA 1 sample as the one loaded into Lane 1 in Fig. 3B) and 1 U DNA gyrase at 37 ℃ for 1 hr.; Lane 3: nicked form of circular DNA (Structure 2 in Fig. 3A) formed from Structure 2 (Fig. 3A) by the action of Nt.BsmAI. This nicked form of DNA was obtained through incubation of a solution that contained 20 mM Tris-acetate, 50 mM potassium acetate, 10 mM magnesium acetate, 1 mM Dithiothreitol, 200 ng of negatively supercoiled circular DNA (Structure 1 in Fig. 3A, the same batch of DNA sample as the one loaded into Lane 2 in Fig. 3B) and 10 U Nt.BsmAI at 37 ℃ for 1 hr; Lane 4: non-G-quadruplex-containing closed circular DNA (Circular DNA 3 in Fig. 3A) genertaed from Structure 2 in Fig. 3A. This non-G-quadruplex-containing relaxed closed circular DNA was obtained through incubation of a solution (50 μl) that contained 50 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM ATP, 10 mM dithiothreitol, 500 ng nicked form of circular DNA (Structure 2 in Fig. 3A, the same batch of DNA sample as the one loaded into Lane 3 in Fig. 3B) and 20 U T4 DNA ligase at 16 ℃ for 8 hrs.

Detailed experimental descriptions of Fig. 4C. Electrophoretic analysis of reactions between G-quadruplex-containing Circular DNA 2 and T7 Endonuclease I. Lane 1: Circular DNA 2 alone;

Lane 2: linear DNA formed from G-quadruplex-containing Circular DNA 2 by the action of T7 Endonuclease I (a type of endonuclease that cleaves non-B structure-containing duplex DNA). This linear DNA was generated through incubation of a solution that contained 50 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM DTT, 200 ng Circular DNA 2 (the same batch of DNA sample as the one loaded into Lane 5 in Fig. 2B) and 5 U T7 Endonuclease I at 37 ℃ for 1 hr.

Detailed experimental descriptions of Fig. 4E. Electrophoretic analysis of reactions between non-G-quadruplex-containing circular DNA (Circular DNA 1) and T7 Endonuclease I. Lane 1: Circular DNA 3 alone; Lane 2: a reaction mixture of non-G-quadruplex-containing Circular DNA 3 and T7 Endonuclease I. This reaction mixture was obtained through incubation of a solution that contained 50 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM DTT, 200 ng Circular DNA 3 (the same batch of DNA sample as the one loaded into Lane 4 in Fig. 3B) and 5 U T7 Endonuclease I at 37 ℃ for 1 hr.

Description of AFM examinations: Immobilization of DNA samples on micas were carried out following the previously reported procedures.[2, 3] AFM images of DNA samples in the current studies were obtained in Tapping Mode™ on a Multimode™ AFM (Veeco, Santa Barbara, CA) in connection with a Nanoscope V™ controller. Antimony (n) doped Si cantilevers with nominal spring constants between 20 and 80 N/m were used and 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. DNA lengths were measured by drawing a series of very short lines along the DNA contour and summating the lengths as described as reported method.[4]

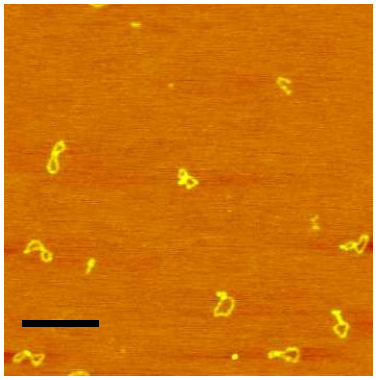

Fig. S1 AFM examination of Structure 1 in Fig. 2A (the DNA sample used for this AFM examination was the same batch of sample as the one loaded in Lane 3 in Fig. 2B). The structures of DNA in the AFM images were G-quadruplex-containing negative supercoils that were generated from relaxed forms of guanine-rich segment-containing circular DNA (Circular DNA 1 in Fig. 2A) under a physiological concentration of potassium ion (150 mM KCl). G-quadruplex structures in these negatively supercoils are not identifiable using AFM owing to their spatial compactness.

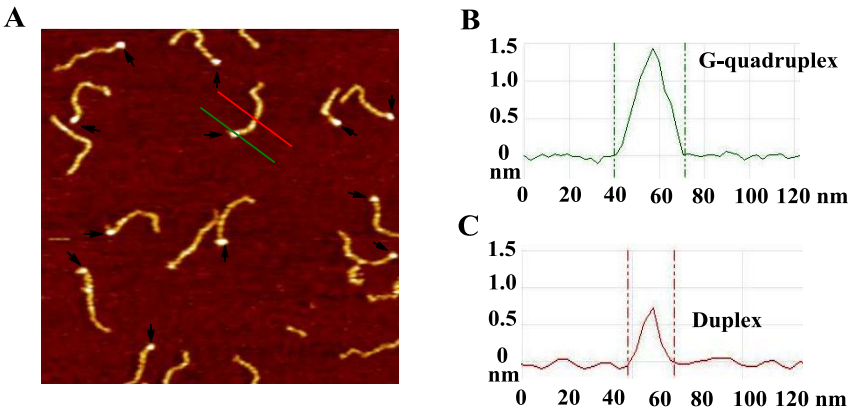

Fig. S2 AFM examination of G-quadruplex-containing Linear DNA 1 [the examinations in the current section serve as a positive control to prove that the character described in Fig. 2 is G-quadruplex]. (A) AFM examination of G-quadruplex-containing Linear DNA 1. The structures of DNA in the AFM images were G-quadruplex-containing Linear DNA 1 that were generated from Linear DNA 1 (the same batch of DNA sample as the one loaded into Lane 1 in Fig. 1B) incubated with 150 mM KCl and 40% PEG 200 at 95^{o}C for 5 min and cooled down to room temperature.[5, 6] (B) Section analysis of end structure of an AFM image in Fig. S2A. (C) Section analysis of duplex part of an AFM image in Fig. S2A.

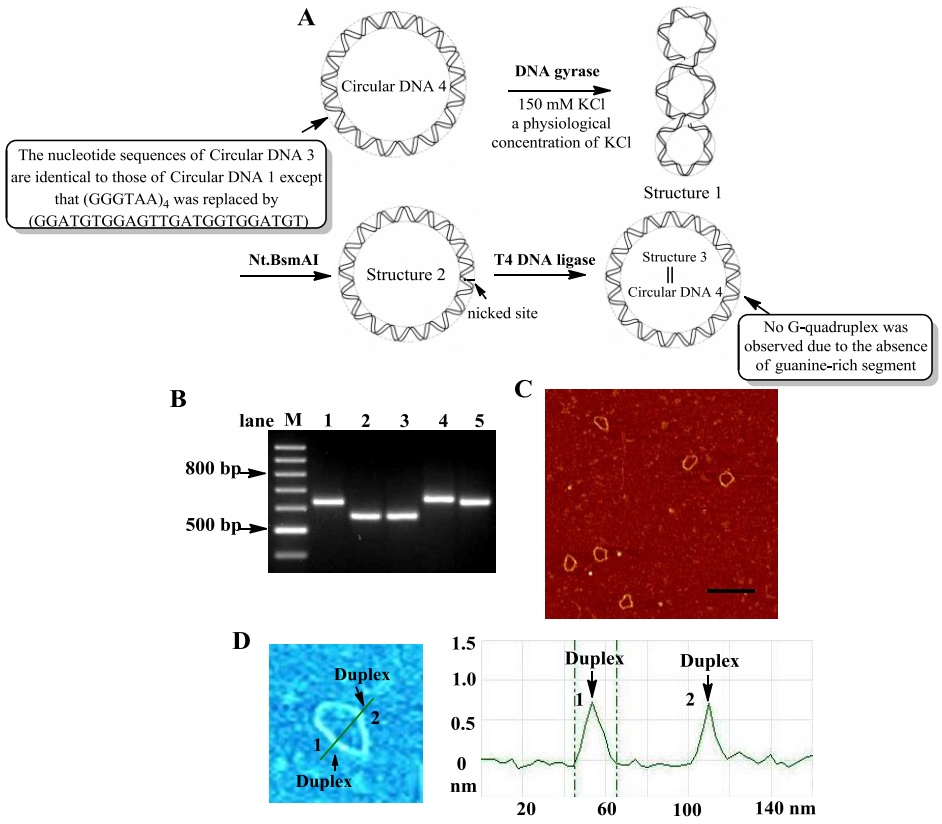

Fig. S3 Examination of action of DNA gyrase on Circular DNA 4 (the nucleotide sequences of Circular DNA 4 are identical to those of Circular DNA 1 except that $(TTAGGG)_4$ was replaced with GGATGTGGAGTTGATGGTGGATGT; the entire nucleotide sequences of Circular DNA 4 are given in Tab. S5) under physiological concentrations of potassium ions [the examinations in the current section (non-guanine-rich-segment-containing circular DNA) serve as control experiments for the studies shown in Fig. 2 (guanine-rich-segment-containing circular DNA)]. A. Illustration of reactions of Circular DNA 4 upon the actions of DNA gyrase, nicking enzyme and DNA ligase. (B) Electrophoretic analysis of products of enzymatic reactions on Circular DNA 4. Lane M: molecular weight markers; Lane 1: Circular DNA 4 alone; Lane 2: negative supercoils of circular DNA generated under a non-physiological concentration of potassium ions (24 mM). This negatively supercoiled DNA was obtained through incubation of a solution (50 μl) that contained 35 mM Tris-HCl, 24 mM KCl (a non-physiological concentration of potassium ion), 4

mM $MgCl_2$, 2 mM DTT, 1.75 mM ATP, 5 mM spermidine, 0.1 mg/ml BSA 6.5% Glycerol, 500 ng Circular DNA 4 (the same batch of DNA sample as the one loaded into Lane 1 in Fig. S3B) and 1 U DNA gyrase at 37 ℃ for 1 hr. Lane 3: negative supercoiled circular DNA (Structure 1 in Fig. S3A) generated under a physiological concentration of potassium ions (150 mM KCl). This negatively supercoiled circular DNA (Structure 1 in Fig. S3A) was obtained through incubation of a solution (50 μl) that contained 35 mM Tris-HCl, 150 mM KCl (a physiological concentration of potassium ions), 4 mM NaCl, 4 mM $MgCl_2$, 2 mM DTT, 1.75 mM ATP, 5 mM spermidine, 0.1 mg/ml BSA, 500 ng Circular DNA 4 (the same batch of DNA sample as the one loaded into Lane 1 in Fig. S3B) and 2 U DNA gyrase at 37 ℃ for 1 hr.; Lane 4: nicked form of circular DNA (Structure 2 in Fig. S3A). This nicked form of DNA was obtained through incubation of a solution that contained 20 mM Tris-acetate, 50 mM potassium acetate, 10 mM magnesium acetate, 1 mM dithiothreitol, 200 ng of negatively supercoiled circular DNA (the same batch of DNA sample as the one loaded into Lane 3 in Fig. S3B) and 10 U Nt.BsmAI at 37 ℃ for 1 hr.; Lane 5: relaxed form of closed circular DNA (Structure 3 in Fig. S3A). This relaxed form of DNA was obtained through incubation of a solution (50 μl) that contained 50 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM ATP, 10 mM dithiothreitol, 500 ng nicked form of circular DNA (the same batch of DNA sample as the one loaded into Lane 4 in Fig. S3B) and 20 U T4 DNA ligase at 16 ℃ for 8 hrs; (C) AFM images of the obtained relaxed form of closed circular DNA with its scale bar of 200 nm. The DNA sample for this AFM examination was the same batch of sample as the one loaded in Lane 5 in Fig. S3B. (D) Section analysis of an AFM image in Fig. S3C.

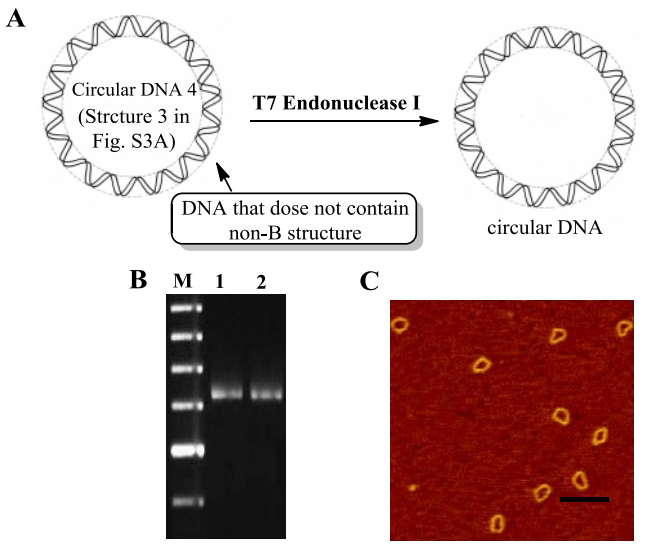

Fig. S4 Enzymatic confirmation of absence of G-quadruplex structures in Circular DNA 4 (Structure 3 in Fig. S3A). (A) Diagrammatic illustration of anticipated reactions of non-G-quadruplex-containing Circular DNA 4 (Structure 3 in Fig. S3) with T7 Endonuclease I. (B) Electrophoretic analysis of reactions of non-G-quadruplex-containing Circular 4 (Structure 3 in Fig. S3) and T7 Endonuclease I. Lane 1: Circular DNA 4 (Structure 3 in Fig. S3, the same batch of DNA sample as the one loaded into Lane 5 in Fig. S3B); Lane 2: a mixture obtained from incubation of Circular DNA 4 with T7 Endonuclease I. This reaction mixture was obtained through incubation of a solution that contained 50 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM DTT, 200 ng Circular DNA 4 (the same batch of DNA sample as the one loaded into Lane 5 in Fig. S3B) and 5 U T7 Endonuclease I at 37 ℃ for 1 hr. (C) AFM images of Circular DNA 4 upon its incubation with T7 Endonuclease I with its scale bar of 200 nm. The DNA sample for this AFM examination was the same batch of sample as the one loaded in Lane 2 in Fig. S4B.

Tab. S1 Nucleotide sequences of Linear DNA 1

```
5' CCGAGCTCAGGATCCGGATGATCCCTAACCCTAACCCTAACCCTAACCAGTCCGTAATACGACTCACTTAAGGCCTTGACTAGAGGGTAC
3' GGCTCGAGTCCTAGGCCTACTAGGGATTGGGATTGGGATTGGGATTGGTCAGGCATTATGCTGAGTGAATTCCGGAACTGATCTCCCATG

CAACCTAGGTATCTAGAACCGGTCTCGAGCCATAACTTCGTATAGCATACATTATACGAAGTTATATAAGCTGTCAAACATGAGAATTCTTG
GTTGGATCCATAGATCTTGGCCAGAGCTCGGTATTGAAGCATATCGTATGTAATATGCTTCAATATATTCGACAGTTTGTACTCTTAAGAAC

TTATAGGTTAATGTCATGATAATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTT
AATATCCAATTACAGTACTATTATTACCAAAGAATCTGCAGTCCACCGTGAAAAGCCCCTTTACACGCGCCTTGGGGATAAACAAATAAAAA

CTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAAC
GATTTATGTAAGTTTATACATAGGCGAGTACTCTGTTATTGGGACTATTTACGAAGTTATTATAACTTTTTCCTTCTCATACTCATAAGTTG

ATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAA
TAAAGGCACAGCGGGAATAAGGGAAAAAACGCCGTAAAACGGAAGGACAAAAACGAGTGGGTCTTTGCGACCACTTTCATTTTCTACGACTT

GATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGTTAAGCTTTTTGCACAACATGGGGGATCATGTAACTCG
CTAGTCAACCCACGTGCTCACCCAATGTAGCTTGACCTAGAGTTGTCGCCATTCAATTCGAAAAACGTGTTGTACCCCCTAGTACATTGAGC

CCTTGATCGAAGGAGAGAAGAGCTGGAGCTCAATGAAGCCATACCAAACGA 3'
GGAACTAGCTTCCTCTCTTCTCGACCTCGAGTTACTTCGGTATGGTTTGCT 5'
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Tab. S2 Nucleotide sequences of Linear DNA 2

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      5'CAGGATCCGGATGATCCCTAACCCTAACCCTAACCCTAACCAGTCCGTAATACGACTCACTTAAGGCCTTGACTAGAGGGTAC
   3'TCGAGTCCTAGGCCTACTAGGGATTGGGATTGGGATTGGGATTGGTCAGGCATTATGCTGAGTGAATTCCGGAACTGATCTCCCATG

CAACCTAGGTATCTAGAACCGGTCTCGAGCCATAACTTCGTATAGCATACATTATACGAAGTTATATAAGCTGTCAAACATGAGAATTCTTG
GTTGGATCCATAGATCTTGGCCAGAGCTCGGTATTGAAGCATATCGTATGTAATATGCTTCAATATATTCGACAGTTTGTACTCTTAAGAAC

TTATAGGTTAATGTCATGATAATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTT
AATATCCAATTACAGTACTATTATTACCAAAGAATCTGCAGTCCACCGTGAAAAGCCCCTTTACACGCGCCTTGGGGATAAACAAATAAAAA

CTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAAC
GATTTATGTAAGTTTATACATAGGCGAGTACTCTGTTATTGGGACTATTTACGAAGTTATTATAACTTTTTCCTTCTCATACTCATAAGTTG

ATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAA
TAAAGGCACAGCGGGAATAAGGGAAAAAACGCCGTAAAACGGAAGGACAAAAACGAGTGGGTCTTTGCGACCACTTTCATTTTCTACGACTT

GATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGTTAAGCTTTTTGCACAACATGGGGGATCATGTAACTCG
CTAGTCAACCCACGTGCTCACCCAATGTAGCTTGACCTAGAGTTGTCGCCATTCAATTCGAAAAACGTGTTGTACCCCCTAGTACATTGAGC

CCTTGATCGAAGGAGAGAAGAGCTGGAGCT 3'
GGAACTAGCTTCCTCTCTTCTCGACC 5'
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Tab. S3 Nucleotide sequences of Circular DNA 1

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<5' GAGCTCAGGATCCGGATGATCCCTAACCCTAACCCTAACCCTAACCAGTCCGTAATACGACTCACTTAAGGCCTTGACTAGAGGGTAC
 3' CTCGAGTCCTAGGCCTACTAGGGATTGGGATTGGGATTGGGATTGGTCAGGCATTATGCTGAGTGAATTCCGGAACTGATCTCCCATG

CAACCTAGGTATCTAGAACCGGTCTCGAGCCATAACTTCGTATAGCATACATTATACGAAGTTATATAAGCTGTCAAACATGAGAATTCTTG
GTTGGATCCATAGATCTTGGCCAGAGCTCGGTATTGAAGCATATCGTATGTAATATGCTTCAATATATTCGACAGTTTGTACTCTTAAGAAC

TTATAGGTTAATGTCATGATAATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTT
AATATCCAATTACAGTACTATTATTACCAAAGAATCTGCAGTCCACCGTGAAAAGCCCCTTTACACGCGCCTTGGGGATAAACAAATAAAAA

CTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAAC
GATTTATGTAAGTTTATACATAGGCGAGTACTCTGTTATTGGGACTATTTACGAAGTTATTATAACTTTTTCCTTCTCATACTCATAAGTTG

ATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAA
TAAAGGCACAGCGGGAATAAGGGAAAAAACGCCGTAAAACGGAAGGACAAAAACGAGTGGGTCTTTGCGACCACTTTCATTTTCTACGACTT

GATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGTTAAGCTTTTTGCACAACATGGGGGATCATGTAACTCG
CTAGTCAACCCACGTGCTCACCCAATGTAGCTTGACCTAGAGTTGTCGCCATTCAATTCGAAAAACGTGTTGTACCCCCTAGTACATTGAGC

CCTTGATCGAAGGAGAGAAGAGCTG 3'>
GGAACTAGCTTCCTCTCTTCTCGAC 5'
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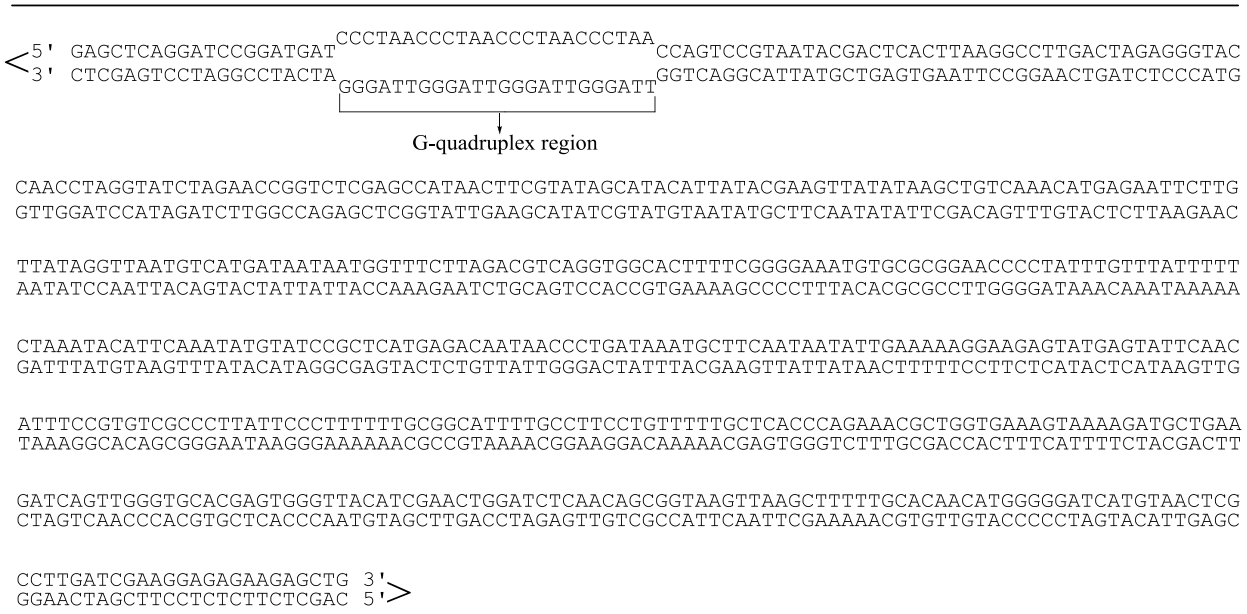
Notes: (1) This DNA is a circular DNA; and

(2) < and > stand for the termini that are covalently connected.

Tab. S4 Nucleotide sequences of Circular DNA 2



Notes: (1) This DNA is a circular DNA; and

(2) < and > stand for the termini that are covalently connected.

Tab. S5 Nucleotide sequences of Circular DNA 4

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<5' GAGCTCAGGATCCGGATGATCCTACACCTCAACTACCACCTACACCAGTCCGTAATACGACTCACTTAAGGCCTTGACTAGAGGGTAC
 3' CTCGAGTCCTAGGCCTACTAGGATGTGGAGTTGATGGTGGATGTGGTCAGGCATTATGCTGAGTGAATTCCGGAACTGATCTCCCATG

CAACCTAGGTATCTAGAACCGGTCTCGAGCCATAACTTCGTATAGCATACATTATACGAAGTTATATAAGCTGTCAAACATGAGAATTCTTG
GTTGGATCCATAGATCTTGGCCAGAGCTCGGTATTGAAGCATATCGTATGTAATATGCTTCAATATATTCGACAGTTTGTACTCTTAAGAAC

TTATAGGTTAATGTCATGATAATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTT
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CTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAAC
GATTTATGTAAGTTTATACATAGGCGAGTACTCTGTTATTGGGACTATTTACGAAGTTATTATAACTTTTTCCTTCTCATACTCATAAGTTG

ATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAA
TAAAGGCACAGCGGGAATAAGGGAAAAAACGCCGTAAAACGGAAGGACAAAAACGAGTGGGTCTTTGCGACCACTTTCATTTTCTACGACTT

GATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGTTAAGCTTTTTGCACAACATGGGGGATCATGTAACTCG
CTAGTCAACCCACGTGCTCACCCAATGTAGCTTGACCTAGAGTTGTCGCCATTCAATTCGAAAAACGTGTTGTACCCCCTAGTACATTGAGC

CCTTGATCGAAGGAGAGAAGAGCTG 3'>
GGAACTAGCTTCCTCTCTTCTCGAC 5'
```

Notes: (1) This DNA is a circular DNA; and

(2) < and > stand for the termini that are covalently connected.

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