Cyclization of secondarily structured oligonucleotides to single-stranded rings by using Taq DNA ligase at high temperatures

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| Name          | Sequences (5’→3’)               | Length (nt) |
|---------------|---------------------------------|-------------|
| splint74(10+9)| ACGTCAAAGGGAGATAGGG             | 19          |
| splint74(11+10)| AACGTCAAAGGGAGATAGGGT         | 21          |
| splint74(12+11)| CAACGTCAAAGGGAGATAGGGTT     | 23          |
| splint74(14+13)| TCCACGTCAAAGGGAGATAGGGGTGA    | 27          |
| splint74(15+14)| CTCACAAGTCAAAGGGAGATAGGGTTGAG | 29          |
| splint74(16+15)| ACTCCAACGTCAAAGGGAGATAGGGGTAGT | 31          |
| splint74(17+16)| GACCTCCAACGTCAAAGGGAGATAGGGGTAGTG | 33         |
| splint74(19+18)| TGGACTCCAACGTCAAAGGGAGATAGGGTTGTTT | 37       |
| splint74(20+19)| GTGGACTCCAACGTCAAAGGGAGATAGGGTTGTTG | 39       |
| l-DNA9GC      | TATTAATATTGGGAGTCCACGTCTTTAAGTGGACTCTTGC | 74         |
| splint9GC(9+8)| TATTAATATTATATATA               | 17          |
| splint9GC(9+9)| TATTAATATTATATATAA              | 18          |
| splint9GC(10+9)| ATATATATATATATATAA          | 19          |
| l-DNA9GC      | TCTTTGACATTGGGAGTCCACGTCTTTAATAGTGGAACACCTTTTGC | 74         |
| splint9GC(9+8)| TGGTACAAGAGAGATAGA              | 17          |
| splint9GC(9+9)| TGGTACAAGAGAGATAGAA             | 18          |
| splint9GC(10+9)| ATGTACAAGAGAGATAGAA          | 19          |
| l-DNA11GC     | TGGCGCTTCTGTTGGGAGTCCACGTCTTATATAGTGGAACACCTTTTGC | 74         |
| splint11GC(9+8)| CGAAAGCAGAGGAGTCCCT         | 17          |
| splint11GC(9+9)| CGAAAGCAGAGGAGTCCCT         | 18          |
| splint11GC(10+9)| AGGAAAGCAGAGGAGTCCCT       | 19          |
| l-DNA11GC     | CGCAGCTCTGTTGGGAGTCCACGTCTGTTTATAGTGGAACACCTTTTGC | 74         |
| splint13GC(9+8)| CAGAGCGGAGAGGTCCCT         | 17          |
| splint13GC(9+9)| CAGAGCGGAGAGGTCCCT         | 18          |
| splint13GC(10+9)| CAGCGGCGGAGAGGTCCCT       | 19          |
| l-DNA13GC     | CCGCGCGCGGTGGGAGTCCACGTCTTAAATAGTGGAACACCTTTTGC | 74         |
| splint17GC(9+8)| CGCGCGCGCGCGCGGCC             | 17          |
| splint17GC(9+9)| CGCGCGCGCGCGCGGCC             | 18          |
| splint17GC(10+9)| CGCGCGCGCGCGCGGCC            | 19          |
| l-DNA17GC     | CTTTGTACGGTGGGAGTCCACGTCTTAAATAGTGGAACACCTTTTGC | 74         |
| splint17GC(9+8)| CTTTGTACGGTGGGAGTCCACGTCTTAAATAGTGGAACACCTTTTGC | 74         |
| splint18GC(9+9)| CTTTGTACGGTGGGAGTCCACGTCTTAAATAGTGGAACACCTTTTGC | 74         |
| splint18GC(10+9)| CTTTGTACGGTGGGAGTCCACGTCTTAAATAGTGGAACACCTTTTGC | 74         |
| l-DNA18GC     | CTTTGTACGGTGGGAGTCCACGTCTTAAATAGTGGAACACCTTTTGC | 74         |
| splint19GC(9+8)| CTTTGTACGGTGGGAGTCCACGTCTTAAATAGTGGAACACCTTTTGC | 74         |
| splint19GC(9+9)| CTTTGTACGGTGGGAGTCCACGTCTTAAATAGTGGAACACCTTTTGC | 74         |
| splint19GC(10+9)| CTTTGTACGGTGGGAGTCCACGTCTTAAATAGTGGAACACCTTTTGC | 74         |
Figure S1. $T_m$ of (A) I-DNA$_{74}$ and (B) I-DNA$_{69}$ were respectively 66.4°C and 65.1°C. High resolution melting (HRM) was used to $T_m$ measurement. The solutions of I-DNAs (1 µM) were prepared in 1× Taq DNA ligase buffer containing EvaGreen (1×). The mixed oligomer solution (10 µL) was pipetted into 96-well microtiter plates and then transferred to a PikoReal Real-Time PCR instrument (Thermo Scientific, Finland). Annealing was performed with a cooling rate of 0.1°C/s from 95°C to 10°C; then, fluorescence dates were collected over a temperature range of 10–95°C in 0.1°C increments (the holding time was 2 seconds). There are at least three parallel tests in one plate.
Figure S2. The ligation of nicked DNA by *Taq* DNA ligase (A). (B) the nicked DNA duplex substrate was formed by two short oligonucleotides (a and b) to a longer complementary oligonucleotide template (19 nt). The short oligonucleotide “a” is 9 nt (L$_{5'-9}$) and “b” is 9 nt (L$_{3'-9}$) or 8 nt (L$_{3'-8}$). Reaction conditions: [L$_{5'-9}$] = 5 μM; [L$_{3'-9}$] = 5 μM (Lanes 1 and 2); [L$_{3'-8}$] = 5 μM (Lanes 3 and 4); [template] = 5 μM; 1× *Taq* DNA ligase buffer at 90°C for 3 min and cooled with ice, then *Taq* DNA ligase (40 U) was added, and the mixture was incubated at 65°C for 12 h. Lanes 1 and 3 without *Taq* DNA ligase are as controls of Lanes 2 and 4.

Sequences of oligonucleotides used here are shown as follows:

Template: CCAGAGGCAGGAGGTCCCG (19 nt)
L$_{5'-9}$: CCGCCTCTG (9 nt)
L$_{3'-9}$: CTCCAGGGC (9 nt)
L$_{3'-8}$: CTCCAGGG (8 nt)
Figure S3. Exonuclease reaction to confirm the ring-structure of product for the cyclization of l-DNA74. The reaction mixtures obtained from l-DNA74 using Taq DNA ligase (A) at 65°C and (B) 70°C with various splints were treated with 20 U Exonuclease I in 1x Exonuclease I buffer at 37°C for 12 h. After the Taq DNA ligase reactions, the mixtures were heated at 85°C for 15 min and analyzed by gel electrophoresis.
Figure S4. Secondary structures of (A) I-DNA_{64}, (B) I-DNA_{54} and (C) I-DNA_{44} obtained by Mfold ([Mg^{2+}] = 10 mM, 25°C).
Figure S5. Time-courses of cyclization of l-DNA$_{74}$ by Taq DNA ligase at 65°C in 1× Taq DNA ligase buffer (A) and in 0.1× Taq DNA ligase buffer (B). [l-DNA$_{74}$] = 5 μM, [splint$_{415^{14}}$] = 10 μM, 40 U Taq DNA ligase(in 20 μL).