Electronic Supplementary Material (ESI) for RSC Advances.

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

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Table S1. The l-DNAs and splints used in Figures 2-5.

Name	Sequences（5′→3′）	Length（nt）
$splint_{74}^{(10+9)}$	ACGTCAAAGGGAGATAGGG	19
$splint_{74}^{(11+10)}$	AACGTCAAAGGGAGATAGGGT	21
$splint_{74}^{(12+11)}$	CAACGTCAAAGGGAGATAGGGTT	23
$splint_{74}^{(14+13)}$	TCCAACGTCAAAGGGAGATAGGGTTGA	27
$splint_{74}^{(15+14)}$	CTCCAACGTCAAAGGGAGATAGGGTTGAG	29
$splint_{74}^{(16+15)}$	ACTCCAACGTCAAAGGGAGATAGGGTTGAGT	31
$splint_{74}^{(17+16)}$	GACTCCAACGTCAAAGGGAGATAGGGTTGAGTG	33
$splint_{74}^{(18+17)}$	GGACTCCAACGTCAAAGGGAGATAGGGTTGAGTGT	35
$splint_{74}^{(19+18)}$	TGGACTCCAACGTCAAAGGGAGATAGGGTTGAGTGTT	37
$splint_{74}^{(20+19)}$	GTGGACTCCAACGTCAAAGGGAGATAGGGTTGAGTGTTG	39
$l\text{-}DNA_{0GC}$	TATTTAATATTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTG GAACAACACTCAATTATATATA	74
$splint_{0GC}^{(9+8)}$	TATTAAATATATATATA	17
$splint_{0GC}^{(9+9)}$	TATTAAATATATATATAA	18
$splint_{0GC}^{(10+9)}$	ATATTAAATATATATATAA	19
$l\text{-}DNA_{6GC}$	TCTTTGACATTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTG GAACAACACTCAATTCTATCTC	74
$splint_{6GC}^{(9+8)}$	TGTCAAAGAGAGATAGA	17
$splint_{6GC}^{(9+9)}$	TGTCAAAGAGAGATAGAA	18
$splint_{6GC}^{(10+9)}$	ATGTCAAAGAGAGATAGAA	19
$l\text{-}DNA_{11GC}$	TCGCTTTCGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACT GGAACAACACTCAAAGGGACCTC	74
$splint_{11GC}^{(9+8)}$	CGAAAGCGAGAGGTCCC	17
$splint_{11GC}^{(9+9)}$	CGAAAGCGAGAGGTCCCT	18
$splint_{11GC}^{(10+9)}$	ACGAAAGCGAGAGGTCCCT	19
$l\text{-}DNA_{15GC}$	CCGCCTCTGGTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACT GGAACAACACTCAACGGGACCTC	74
$splint_{13GC}^{(9+8)}$	CAGAGGCGGGAGGTCCC	17
$splint_{14GC}^{(9+9)}$	CAGAGGCGGGAGGTCCCG	18
$splint_{15GC}^{(10+9)}$	CCAGAGGCGGGAGGTCCCG	19
$l\text{-}DNA_{19GC}$	CCGCCGCCGGTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAAC TGGAACAACACTCAACGGGCCCGC	74
$splint_{17GC}^{(9+8)}$	CGGCGGCGGGCGGGCCC	17
$splint_{18GC}^{(9+9)}$	CGGCGGCGGGCGGGCCCG	18
$splint_{19GC}^{(10+9)}$	CCGGCGGCGGGCGGGCCCG	19
$l\text{-}DNA_{64}$	CCTTTGACGTTGGAGTCCACGTTCTTTAATAGTTCCAAACTGGAACAACAC TCAACCCTATCTC	64
$l\text{-}DNA_{54}$	CCTTTGACGTTGGAGTCCACGTTCTTTAACTGGAACAACACTCAACCCTAT CTC	54
$l\text{-}DNA_{44}$	CCTTTGACGTTGGAGTCCACGTGAACAACACTCAACCCTATCTC	44

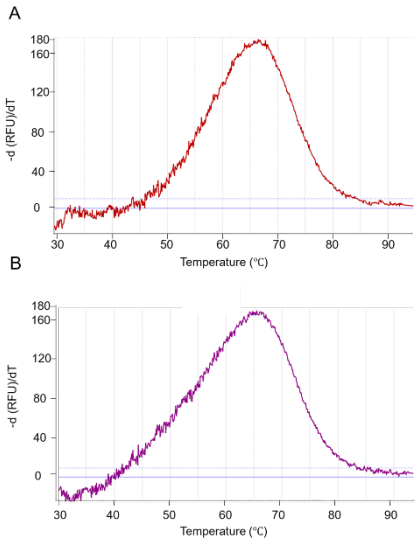

Figure S1. T_m of (A) l-DNA$_{74}$ and (B) l-DNA$_{59}$ were respectively 66.4°C and 65.1°C. High resolution melting (HRM) was used to T_m measurement. The solutions of l-DNAs (1 μM) were prepared in 1× *Taq* DNA ligase buffer containing EvaGreen (1×). The mixed oligomer solution (10 μL) was pipetted 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.

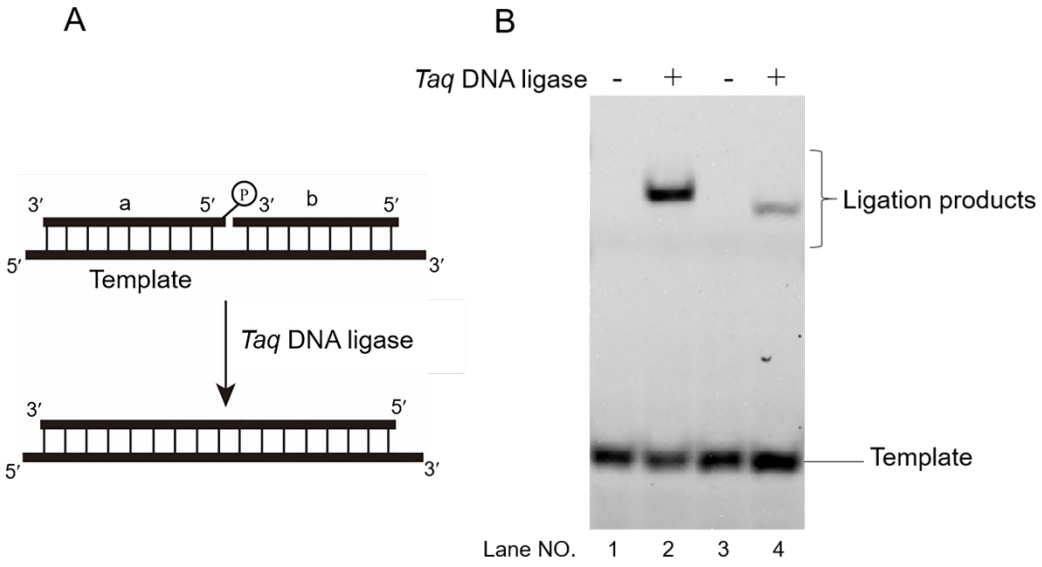

Figure S2. The ligation of nicked DNA by *Taq* DNA ligase (A). (B) the nicked DNA duplex substrate was formed by two short oligonucleotides (a and b) to a longer complementary oligonucleotide template (19 nt). The short oligonucleotide "a" is 9 nt ($L_{5'\text{-}9\ nt}$) and "b" is 9 nt ($L_{3'\text{-}9nt}$) or 8 nt ($L_{3'\text{-}8nt}$). Reaction conditions: [$L_{5'\text{-}9\ nt}$] = 5 μM; [$L_{3'\text{-}9nt}$] = 5 μM (Lanes 1 and 2); [$L_{3'\text{-}8nt}$] = 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: CCAGAGGCGGGAGGTCCCG (19 nt)

$L_{5'\text{-}9\ nt}$: CCGCCTCTG (9 nt)

$L_{3'\text{-}9nt}$: CTCCAGGGC (9 nt)

$L_{3'\text{-}8nt}$: CTCCAGGG (8 nt)

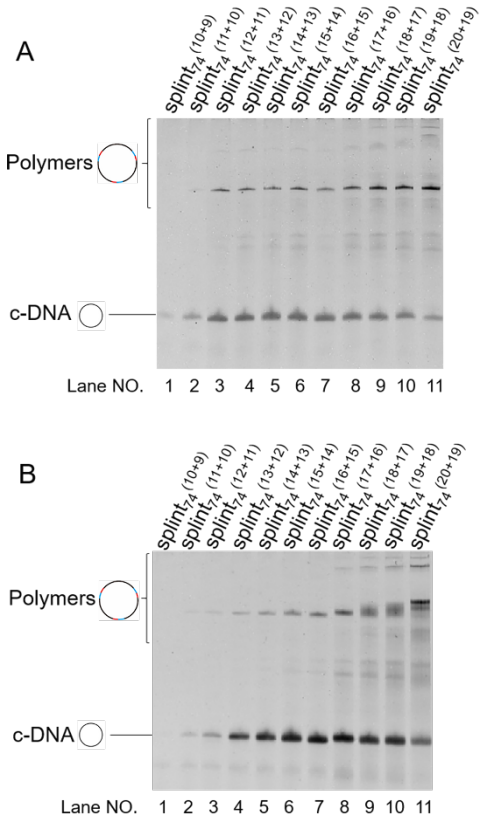

Figure S3. Exonuclease reaction to confirm the ring-structure of product for the cyclization of l-DNA$_{74}$. The reaction mixtures obtained from l-DNA$_{74}$ using *Taq* DNA ligase (A) at 65°C and (B) 70°C with various splints were treated with 20 U Exonuclease I in 1x Exonuclease I buffer at 37°C for 12 h. After the *Taq* DNA ligase reactions, the mixtures were heated at 85°C for 15 min and analyzed by gel electrophoresis.

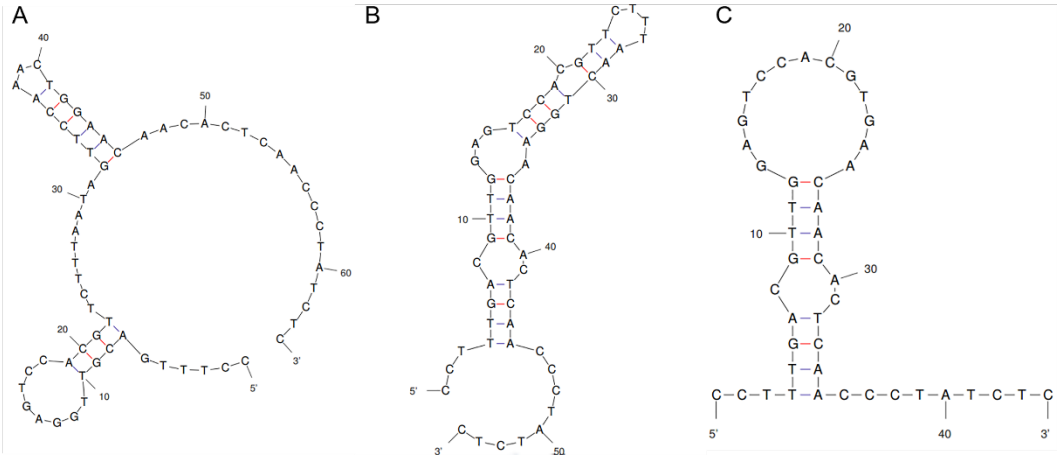

Figure S4. Secondary structures of (A) l-DNA$_{64}$, (B) l-DNA$_{54}$ and (C) l-DNA$_{44}$ obtained by Mfold ([Mg^{2+}] = 10 mM, 25°C).

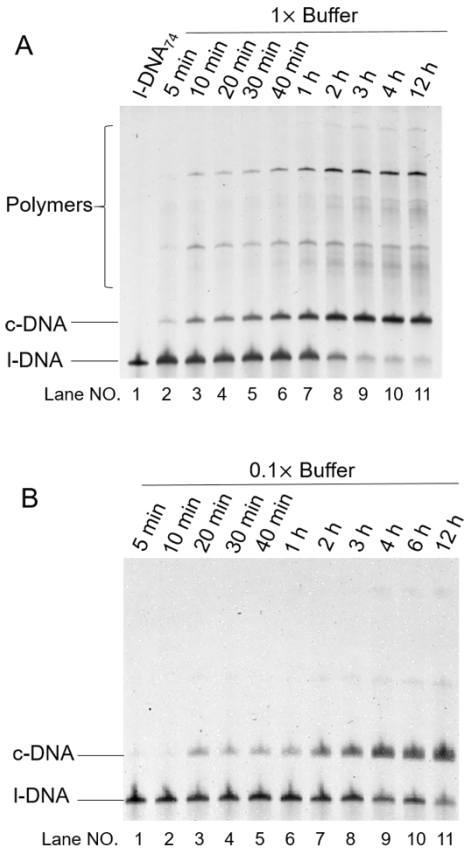

Figure S5. Time-courses of cyclization of I-DNA$_{74}$ by *Taq* DNA ligase at 65°C in 1× *Taq* DNA ligase buffer (A) and in 0.1× *Taq* DNA ligase buffer (B). [I-DNA$_{74}$] = 5 μM, [splint$_{74}^{(15+14)}$] = 10 μM, 40 U *Taq* DNA ligase(in 20 μL).