Supporting Information

A Replicable Tetrahedral Nanostructure Self-Assembled from a Single DNA Strand

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Materials. All DNA oligonucleotides were purchased from Integrated DNA Technology and purified by polyacrylamide gel electrophoresis (PAGE). Restriction enzymes (PstI, SacI, BsrGI, and BspHI), T4 DNA ligase, phagemid vector Litmus 28I, and helper phage M13KO7 were purchased from New England BioLabs. Competent cell line XL1-Blue was purchased from Stratagene. 10-bp DNA ladders were purchased from Invitrogen. Plasmid Spin Miniprep kit was purchased from Qiagen. All other reagents were purchased from Sigma–Aldrich.

***In vivo* cloning.** The process is illustrated schematically in Fig S3.

1) Preparation of double stranded insert and ligation with the plasmid: Equal amounts of sense and antisense nanostructure strands (90 nM, sequences are listed in Table S1 and S2 respectively) were annealed in a water bath from 95 °C to room temperature for about 48 hours in 1X TAE/Mg^{2+} buffer to yield a double-stranded (DS) insert. 2 μg Litmus 28i (500 μg/mL) were digested by 20 U PstI and 15 U SacI in 50 μL 1X NEBuffer 1 at 37 ºC

for 2 hrs, and purified via agarose gel electrophoresis. 100 ng of digested vector was ligated with 0.16 pmol of pre-annealed DS insert (~ 3 fold excess) in 20 μL 1X T4 ligase buffer at 4 ºC overnight.

2) Transformation of the cell by the ligated vector and verification of the correct vector insertion: The ligated vector, 50 ng, was transformed into competent XL1-Blue cells by heat shock, and incubated on LB-ampicillin (LB-Amp) agar plates at 37 ºC overnight. Double stranded phagemid was extracted using the plasmid miniprep kit from cells in 5 mL of saturated cultures that were amplified from a single colony picked from the agar. The correct insertion was verified by restriction enzyme digestion followed by denaturing PAGE.

3) Infection of the transformed cells with helper phage, amplification and packaging of the single stranded phage DNA: 1 mL glycerol stock of XL1-Blue cells (OD_{600}=0.8) with correctly inserted phagemid were infected by 50 μL of $1x10^{11}$ M13KO7 helper phage and incubated overnight at 37 ºC in 250 mL LB-Amp culture containing 25 μg/mL Kanamycin.

4) Isolation and purification of the amplified single stranded tetrahedron DNA: The bacteriophage particles that contained single-stranded vectors were precipitated from the supernatant by addition of 10 g PEG and 7.5 g NaCl followed by centrifugation at 10,000 g. Protein shells were removed from the single-stranded vectors by phenol/chloroform extraction. DNA was recovered by ethanol precipitation, re-dissolved in 0.9 mL water, and restricted by 500 U of PstI and 360 U of SacI in the presence of 1 nmol of restriction helper strands in 1 mL 1X NEbuffer 1. The digested single-stranded vector was resolved

on a 10% denaturing polyacrylamide gel and the correctly replicated insert was excised from the gel and eluted. Typically, about 50 pmol of ssDNA was recovered.

TEM imaging. Carbon-coated grids (400 mesh, Ted pella) were glow discharged using an Emitech K100X machine, before the deposition of DNA samples (3 μL). Excess samples were wicked from the grid with a piece of filter paper. The grid was then washed with water by touching it quickly with a drop of water and wicking out the excess with filter paper. The grid was touched with a drop of 0.7 % uranyl formate solution and excess solution was wicked away with a filter paper. Again the grid was touched with the second drop of uranyl formate solution for 15 seconds, and the excess solution was removed with a filter paper. To evaporate extra solution, the grid was kept at room temperature. TEM images were collected by Dr. Zhengtao Deng using a JEOL JEM 2010F transmission electron microscope, operated at 200 kV in the bright field mode.

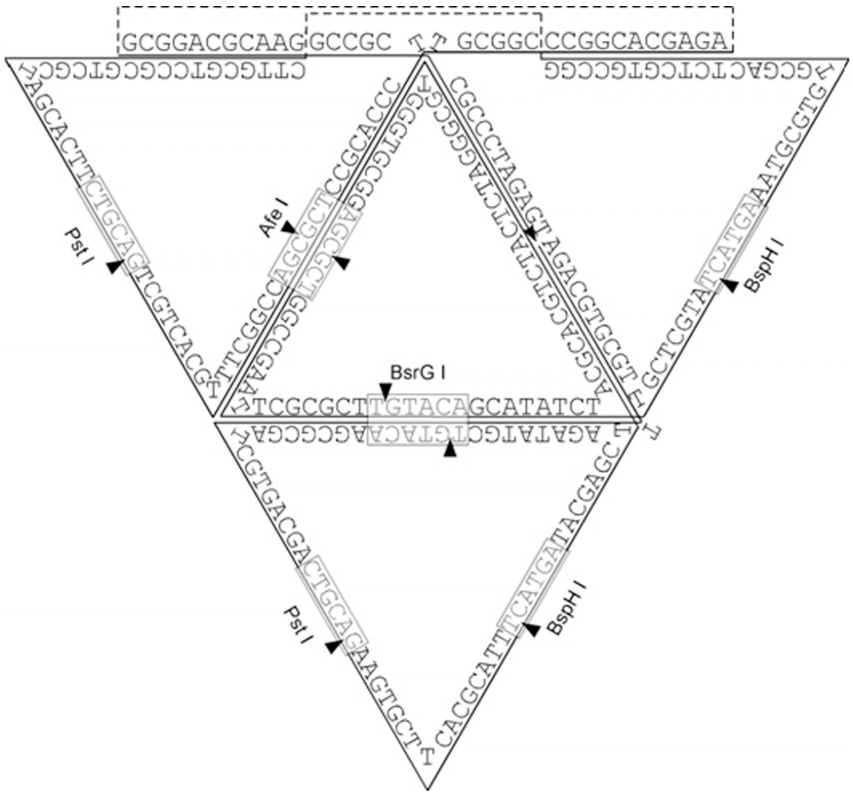

Fig S1. DNA sequence of the single-stranded tetrahedron.

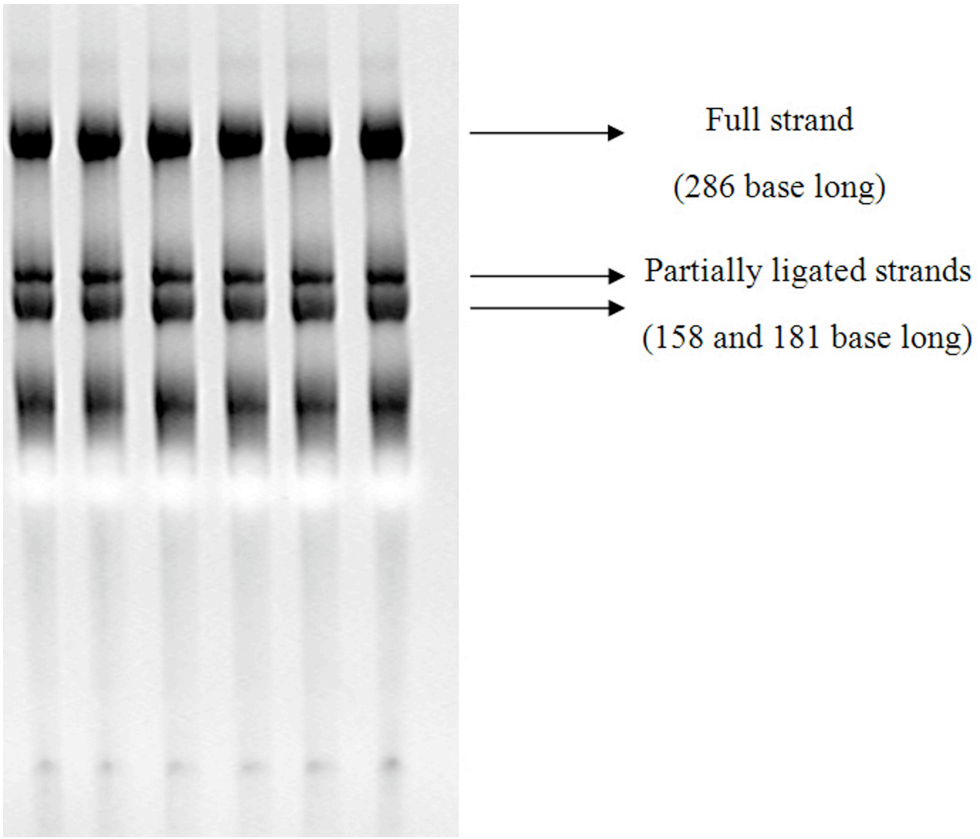

Figure S2. Purification of the 286-nt full length strand obtained by ligation. The gel band of full strand was excised out and extracted for 48 hours in the extraction buffer.

Table S1. Sequences of component strands of the DNA tetrahedron for sense ligation.

Sense component strand 1*	/Phos/CAGACGTGCGTTAGATATGCTGTACAAGCGC GATCGTGACGACTGTAGAAGTGCTTCACGCATTTC ATGATACGAGCTACGCACGTCTACTCTAGGGCGTG GGTGCGGAGCGCTGG
Sense component strand 2*	/Phos/CCGAATTCGCGCTTGTACAGCATATCTTGCTC GTATCATGAAATGCGTGTGCGACTCTCGTGCCGGC TTGCGTCCGCGTCGCTAGCACTTCTACAGTCGTCAC GTTTCGGCCAG
Sense component strand 3*	/Phos/CGCTCCGCACCCTGCGGCCCGGCACGAGAGC GGACGCAAGGCCGCTCGCCCTAGAGTCTGCA
Splint between sense strand 1&2	CTGTACAAGCGCGAATTCGGCCAGCGCTCCGCACC CACGC
Splint between sese strand 2&3	GGGCCGCAGGGTGCGGAGCGCTGGCCGAAACGTG ACGACT

* The 5' end of the strand is phosphorylated.

Table S2. Sequences of constituent strands for antisense ligation.

Antisense component strand 1*	/Phos/GACTCTAGGGCGAGCGGCCTTGCGTCCGCTCT CGTGCCGGGCCGCAGGGTGCGGAGCGCTGGCCGA AACGTGACGACTGTAGAAGTGCTAGCGACGCGGA CGCAAGCCGGCACGAG
Antisense component strand 2*	/Phos/AGTCGCACACGCATTTCATGATACGAGCAAG ATATGCTGTACAAGCGCGAATTCGGCCAGCGCTCC GCACCCACGCCCTAGAGTAGACGTGCGTAGCTCGT ATCATGAAATGCG
Antisense component strand 3*	/Phos/TGAAGCACTTCTACAGTCGTCACGATCGCGCT TGTACAGCATATCTAACGCACGTCTGAGCT
Splint between antisense strand 1&2	CATGAAATGCGTGTGCGACTCTCGTGCCGGCTTGC GTCCG
Splint between antisense strand 2&3	ACGACTGTAGAAGTGCTTCACGCATTTCATGATAC GAGCT

* The 5' end of the strand is phosphorylated.

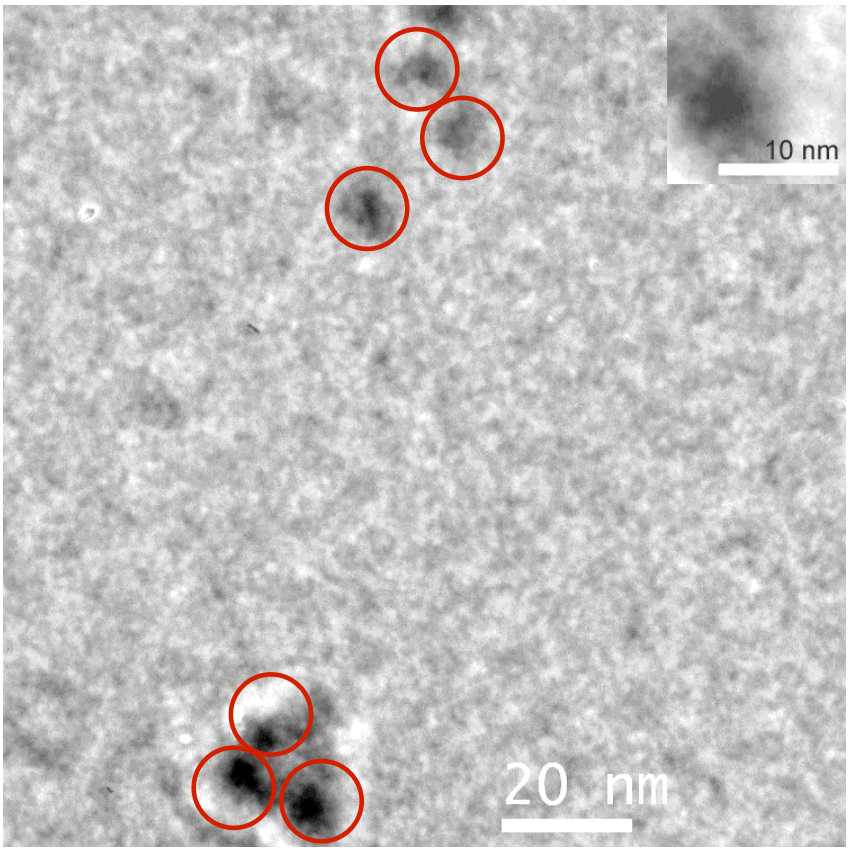

Figure S3. TEM images of ss-tetrahedron DNA structures. Individual tetrahedra are circled. The sizes of the triangle-shaped structure are in agreement with the design (~7.5 nm).

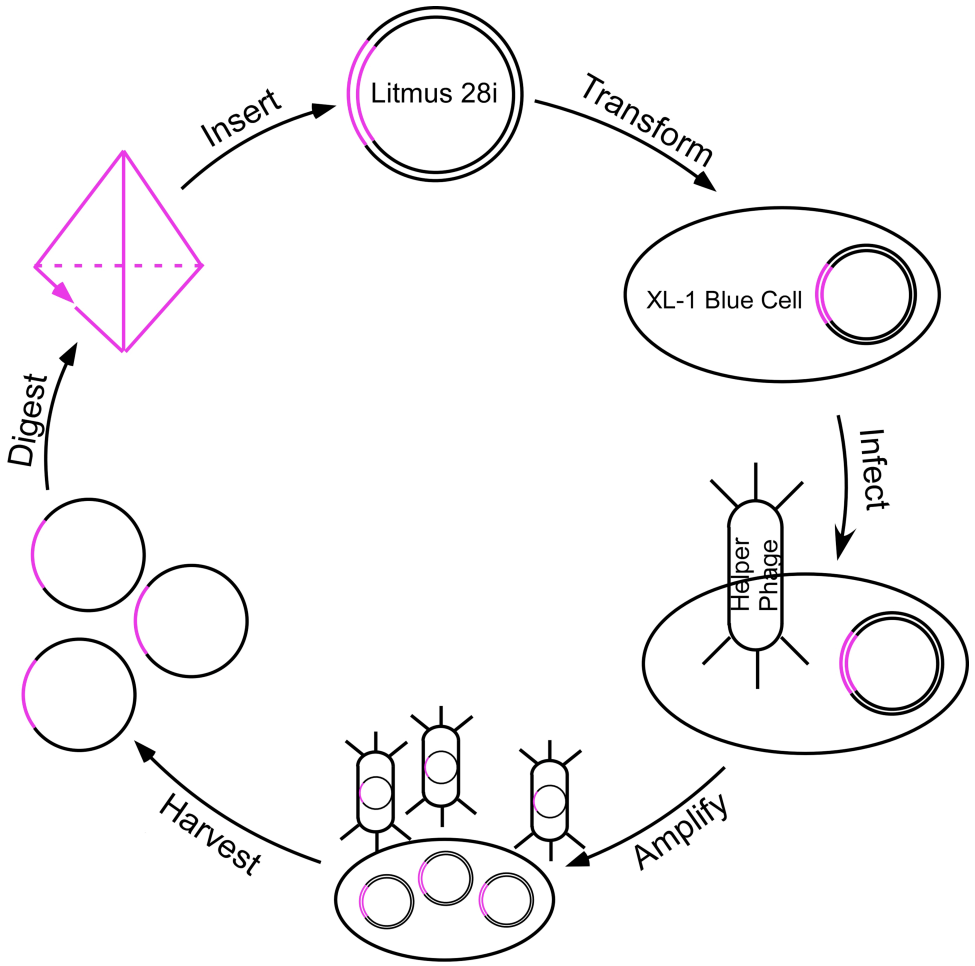

Fig S4. Scheme showing the *in vivo* replication process of the single-stranded DNA tetrahedron.

Rolling circle amplification of the tetrahedron. Rolling circle amplification (RCA) [S1] was attempted to propagate this strand (Fig S4). A total 20-mer loop with Sac I recognition site was designed to extend out from one edge of the tetrahedron (Fig S5). The 5' and the 3' ends in the loop region were covalently connected by CircLigase (Epicentre Biotech). The circularized strand served as the original template for the rolling circle amplification. Phi 29 DNA polymerase then repeatedly read through the circular template for amplification after the 20-mer primer was annealed to the loop region. As a result, a long ssDNA that was composed of repetitive anti-sense strand segments was obtained. After restriction digestion by Sac I enzyme, the product was analyzed by denaturing PAGE. However, as seen in Fig S6, this method did not result in the efficient amplification of the desired structure.

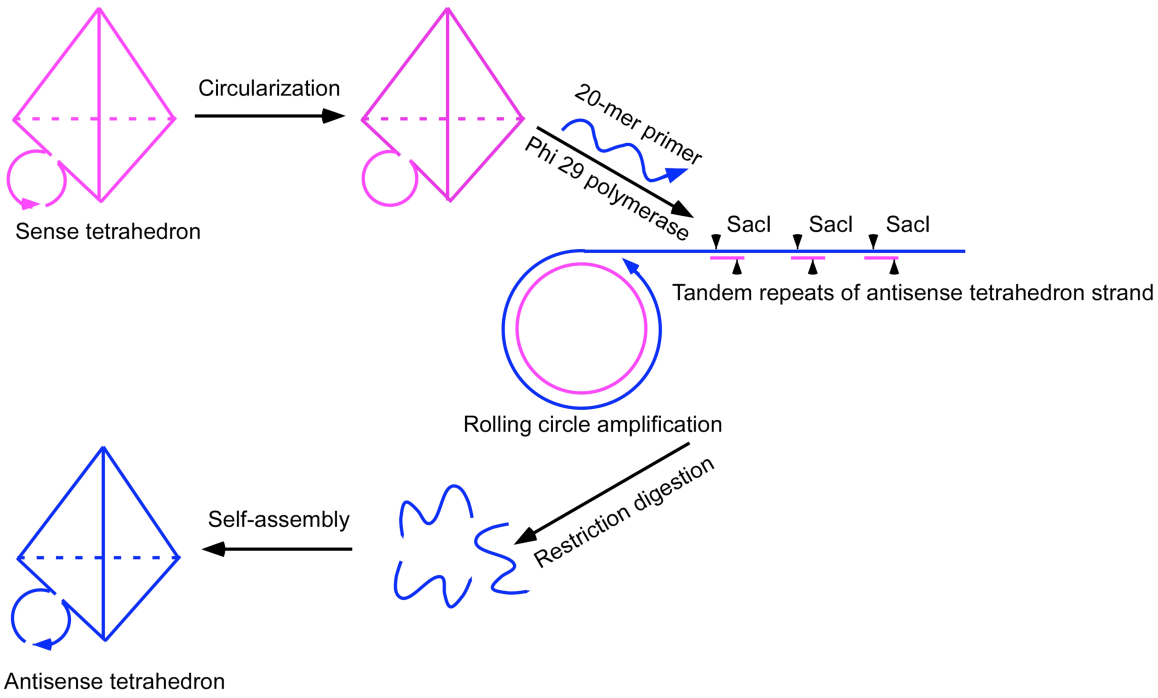

Fig S5. Schematic of rolling circle amplification of DNA tetrahedron.

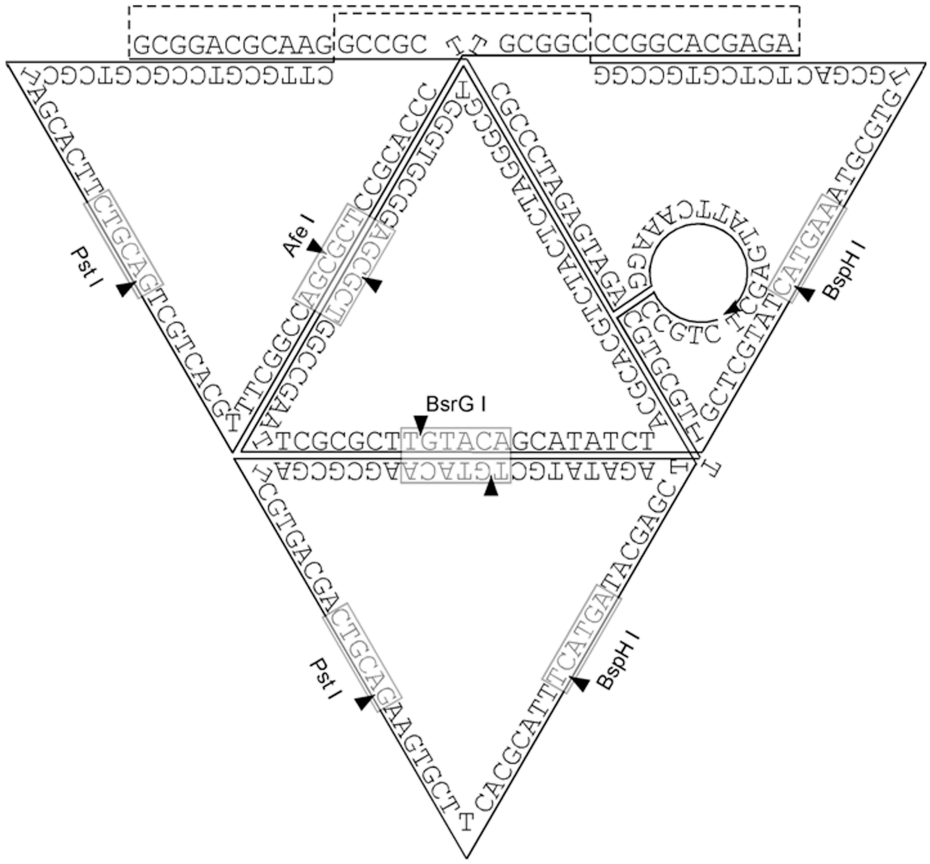

Fig S6. DNA sequence of the single-stranded template for RCA.

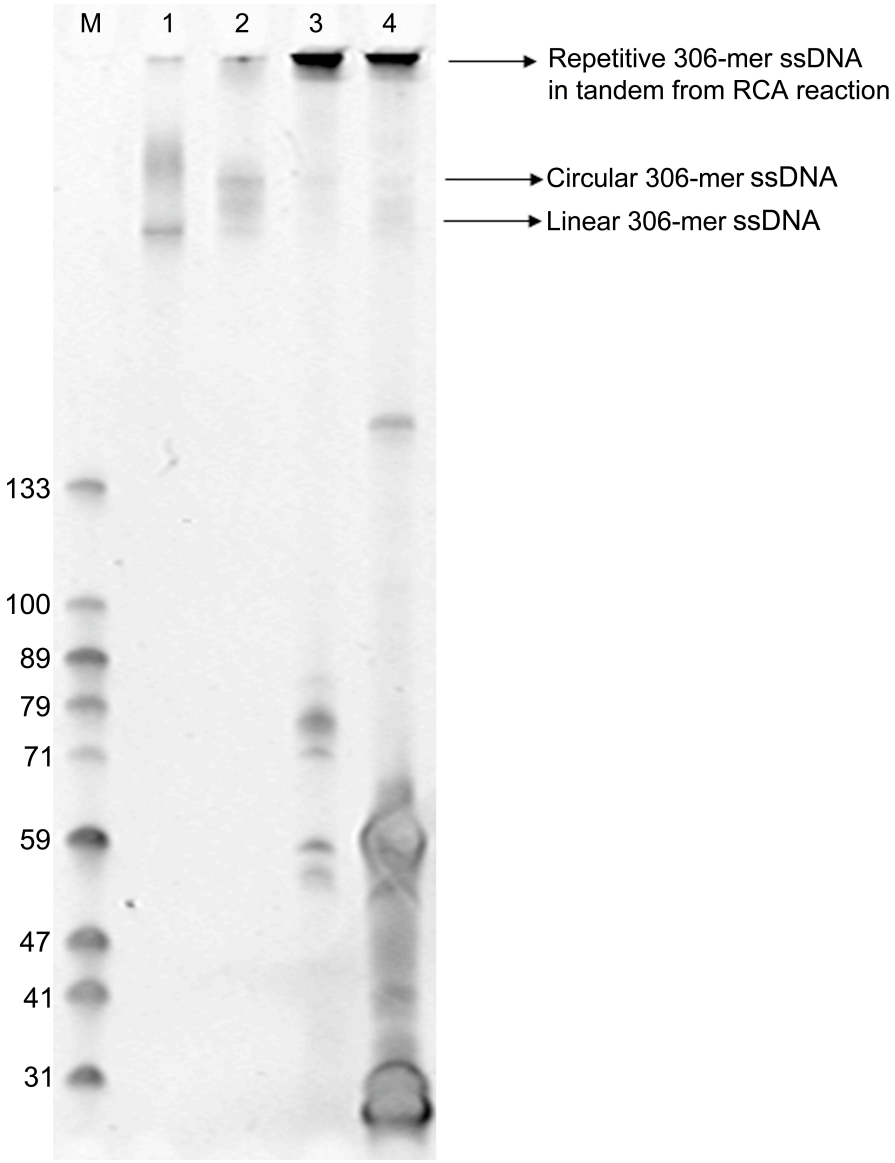

Figure S7. RCA result visualized by denaturing PAGE. ssDNA markers were loaded in lane M. The linear 306-mer ssDNA was loaded in lane 1. Circular 306-mer template (cyclized by CircLigase) was loaded in lane 2. RCA product and its SacI digested product were loaded in lane 3 and 4 respectively. The intensity of the linear 306-mer band was expected to be much higher in lane 4 than in lane 1 if the amplification was successful. The result was opposite with mostly truncated products, indicating extremely low yield of the full length product of this procedure.

Reference

[S1] C. Lin, M. Xie , J. J. L. Chen, Y. Liu, H. Yan, *Angew. Chem*. **2006**, 45, 7537- 7539.