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Electronic Supplementary Information

Periodical Assembly of Repetitive RNA Sequences Synthesized by Rolling Circle Transcription with Short DNA Staple Strands to RNA/DNA Hybrid Nanowires

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Material and Methods

The oligonucleotide strands were purchased from Sangon Biotech (Shanghai) Co. Ltd. and purified by 20% denaturing PAGE. T4 DNA ligase and Exonuclease I were purchased from Takara Biotechnology Co., Ltd (Dalian, China). Water used in all experiments was treated as follows: to eliminate the RNase contamination, add 0.1% DEPC (diethylpyrocarbonate) to the Milli-Q ultrapure water, mix, and sit at room temperature over night, then autoclave at 121 ^{0}C for 20 min. All of the glassware, eppis and pipette tips were also sterilized by autoclaving at 121 ^{0}C for 20 min.

1. Preparation of Long Single-Stranded Repetitive RNAs

1.1 Preparation of circular templates

A 100 μL solution of 96 nt 5'-phosphorylated, linear oligonucleotide (3.5 μM) and its corresponding splint (4.5 μM) in 20 mM TE buffer (Tris-EDTA(ethylene diamine tetraacetic acid), 100 mM NaCl, pH 7.8), was heated to 95 ^{0}C, then cooled down slowly to room temperature. T4 ligase (350 U/μL, 10 μL) and 10×ligase buffer (10 μL) were added and the mixture was incubated for 16 h at 16 ^{0}C. Then, the ligase was inactivated by heating at 65 ^{0}C for 10 min. After the ligation and inactivation step, 15 μL Exonuclease I (5U/μL) and 13 μL 10×Exonuclease I buffer were added to digest the remaining linear DNA template and its corresponding splint by incubation at 37 ^{0}C for 30 min. The circular DNA template was purified by phenol/chloroform/isoamyl alcohol (v: v: v, 25:24:1) abstraction, ethanol precipitation and vacuum drying.

1.2 Rolling circle transcription reaction

The long single-stranded repetitive RNA molecules were prepared in vitro by RiboMAX™ Large Scale RNA Production System—T7 (Promega, USA) from their corresponding circular DNA templates. Following the technical bulletin, all transcription reaction components were combined together at room temperature and incubated at 37 ^{0}C for 2~4 h, with a total volume of 20 μL, including 1 μg DNA template, 4 μL of 5 × T7 RNAP reaction buffer, 2 μL of each 100 mM rNTP, 2 μL of 100 mM DTT, and 2 μL of T7 RNAP. After transcription, DNA templates were removed by DNase I digestion and RNA was extracted with 1 volume of citrate-saturated phenol (pH 4.7): chloroform: isoamyl alcohol = 125:24:1. Then, RNA molecules were precipitated by ethanol. Note: wearing mouth mask and latex-gloves is a must for RNA operation, since any improper operation

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could lead to RNA degradation, and the longer the RNA chain, the easier to degrade.

2. Nucleic Acid Sequences

2.1 Nucleic acid sequences for preparing circular DNA templates

S_1: 5’- PO_3 -TAA GAT GAA GAT AGC GCA CAA TGG TCG GAT TCT CAA CTC GTA TTC TCA ACT CGT ATT CTC AAC TCG TCT CTG CCC TGA CTT CTA TGC CCA GCC CTG-3’

The corresponding splint: 5’- CTT CAT CTT ACA GGG CTG GG-3’

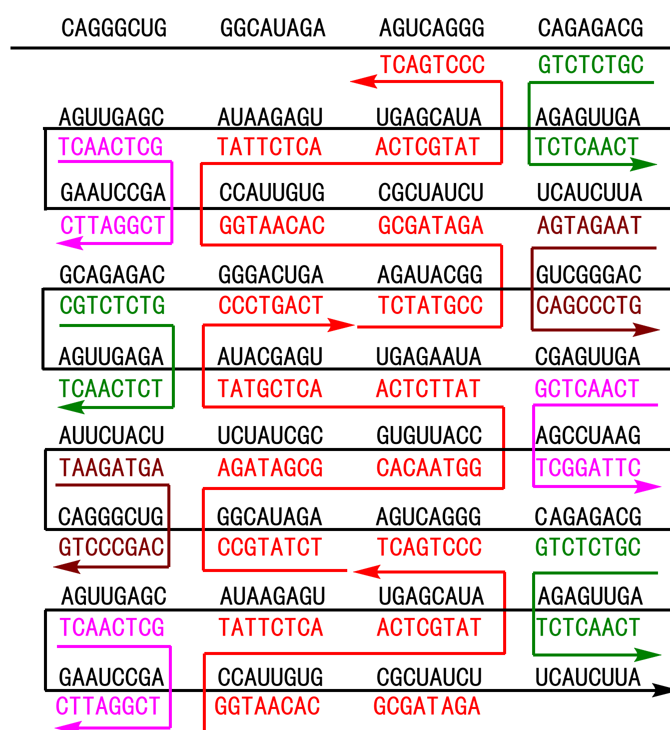
S_2: 5’-PO_3-CTC AGC TGT GAT CAT ACT ATG CTA GTC CTG TAT GTC ATG CCG TTG TGC CTG AGC ACC AGT CGG CAG TCG CAC GAC CTG GCG TTC GCA TGT CCT ATC-3’

The corresponding splint: 5’-ATG ATC ACA GCT GAG GAT A-3’

2.2 Nucleic acid sequences for creating nanowires

2.2.1 R1-1origami sequence codes.

Staple (colorful)	Sequence
red	TCTATGGC AGATAGCG CACAATGG TATTCTCA ACTCGTAT CCCTGACT
green	CGTCTCTG TCTCAACT
rose	TCAACTCG TCGGATTC
brown	TAAGATGA CAGCCCTG



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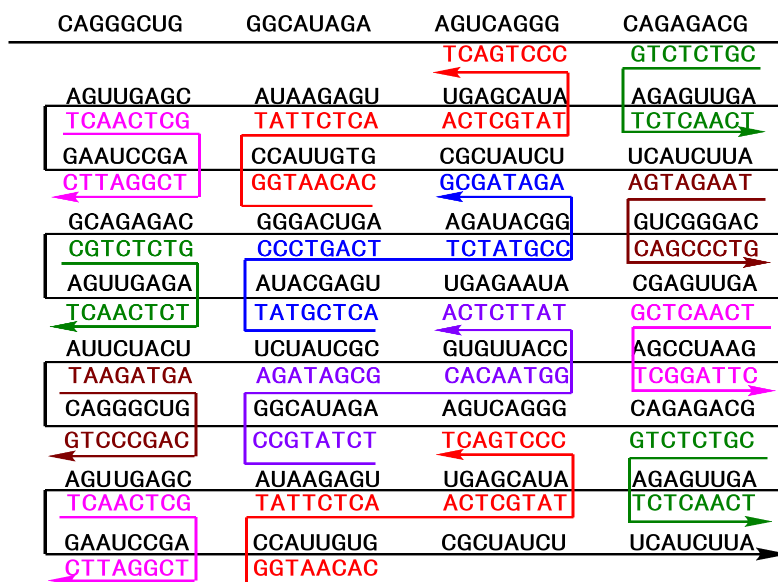
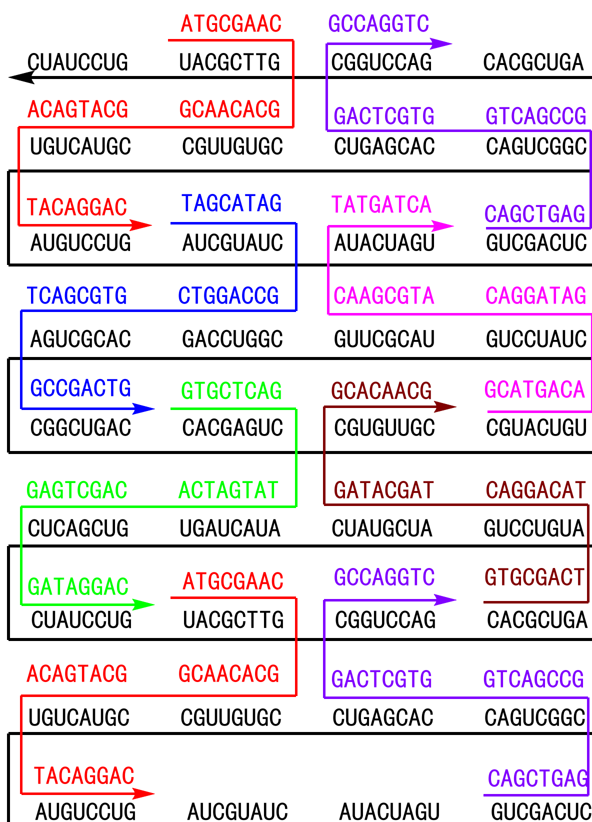
2.2.2 R1-2 origami sequence codes.

Staple (colorful)	Sequence
red	CACAATGGTATTCTCAACTCGTATCCCTGACT
blue	ACTCGTATCCCTGACTTCTATGCCAGATAGCG
purple	TCTATGCCAGATAGCGCACAATGGTATTCTCA
rose	TCAACTCGTCGGATTC
green	CGTCTCTGTCTCAACT
brown	TAAGATGACAGCCCTG



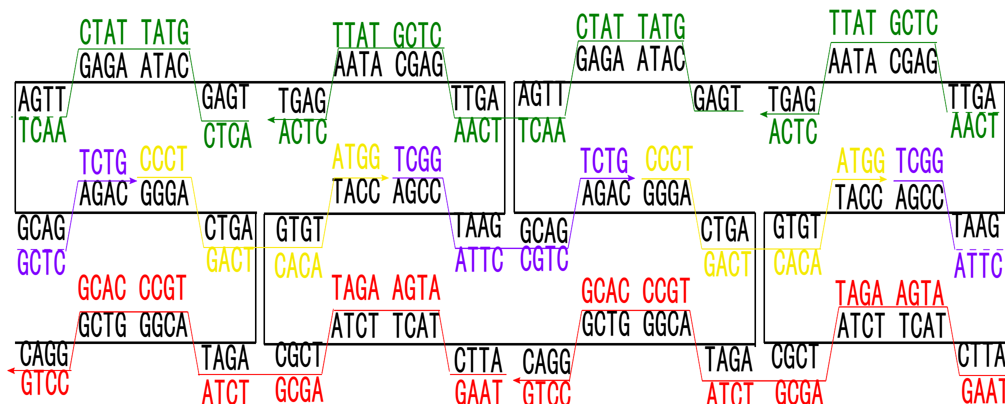
2.2.3 R2-3 origami sequence codes.

Staple (colorful)	sequence
red	ATGCGAACGCACAACGGCATGACATACAGGAC
green	GTGCTCAGTATGATCACAGCTGAGGATAGGAC
blue	TAGCATAGGCCAGGTCGTGCGACTGCCGACTG
rose	GCATGACAGATAGGACATGCGAACTATGATCA
brown	GTGCGACTTACAGGACTAGCATAGGCACAACG
purple	CAGCTGAGGCCGACTGGTGCTCAGGCCAGGTC



2.2.4 R1-4 origami sequence codes.

Staple (colorful)	Sequence
red	ACTCGTATTCTCAACTTCAACTCGTATTCTCA
purple	CCCTGACTCACAATGG
yellow	TCGGATTCCGTCTCTG
green	TAAGATGAAGATAGCGTCTATGCCCAGCCCTG



3. Argrose Electrophoresis

Gel electrophoresis was performed on a 1% agarose gel with 1×TAE (Tris-acetate-EDTA, pH 8.3) as the gel and running buffer.

Gels were run at 13 V/cm for 1.5 h.

Gels were stained using the gel-red (Biotium Inc., USA).

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The transcripts were loaded after mixing with 6×loading buffer and 50% formamide.

4. Thermal Annealing to Create RNA/DNA Nanowires

The scaffold RNA and the short staple DNA strands with the matching molar ratios were mixed to a concentration of 0.1~0.5 μM in a volume of 20 μL 1× TAE/Mg^{2+} buffer, consisting of 40 mM Tris (pH 7.6), 20 mM acetic acid, 2 mM EDTA and 25 mM magnesium acetate. The sample was cooled from 95 ^{0}C to 4 ^{0}C in a PCR machine at a rate of 1 ^{0}C /100s.

5. AFM Imaging

AFM was performed in the tapping mode in air. An annealed sample (5 μL) was dropped onto freshly cleaved mica and stayed there for 2 min, soaked up with a filter paper, washed with 50 μL water two times, and dried with N_2. AFM images were obtained on a Multimode V instrument by using NP-S oxide-sharpened silicon nitride tips (Bruker).