A Simple DNA-Based Translation System

Alejandra V. Garibotti, Shiping Liao and Nadrian C. Seeman*

SUPPLEMENTARY MATERIAL

This supplementary material contains two parts. The first part is the experimental methods used to conduct the experiments reported. The second part consists of sequencing traces of the translated products.

EXPERIMENTAL METHODS

Sequence Design

The sequences have been designed by applying the principles of sequence symmetry minimization, using the program SEQUIN.[1,2]

Synthesis and Purification

The strands were either synthesized on an Applied Biosystem 394 or an Expedite 8909, removed from the support, and deprotected using routine phosphoramidite procedures.[3] Additional strands were purchased from IDT (Coralville, IA). Strands were purified using denaturing gel electrophoresis. Gels were stained with ethidium bromide, and the target band was excised and eluted in a solution containing 500 mM ammonium acetate, 10 mM magnesium acetate, and 1 mM EDTA. The eluates were subjected to extraction with n-butanol to remove ethidium bromide, followed by ethanol precipitation.

Formation of Hydrogen-Bonded Complexes and Arrays

Complexes were formed by mixing a stoichiometric quantity of each strand as estimated by OD_{260}. Concentration of DNA and buffer conditions varied. Mixtures were annealed from 90°C to room temperature during 40 h in a 2-litre water bath insulated in a styrofoam box. 'Fast annealing' consists of incubating the sample 5 min at 90ºC, 15 min at 65ºC, 20 min at 45ºC, 20 min at 37ºC and 30 min at room temperature.

Non-Denaturing Gel Electrophoresis

Annealed complexes, were run on non-denaturing gels to check for tile formation and stoichiometry. The systems were annealed at various DNA concentrations (0.1- 3 μM) in 40 mM Tris•HCl, 20 mM acetic acid, 125 mM Mg Acetate, 2 mM EDTA. Tracking dye containing buffer, 50% glycerol, and a trace amount of Bromphenol Blue and Xylene Cyanol FF was added to the annealed sample before loading them on 6-8% acrylamide gels, containing their respective buffer. Gels were run on a Hoefer SE-600 gel electrophoresis unit at room temperature, with the respective running buffer. After electrophoresis, the gels were stained with ethidium bromide.

Denaturing Gel Electrophoresis

Gels contain 5-20% acrylamide (19:1, acrylamide:bisacrylamide). These gels contain 8.3 M urea. The running buffer consists of 89 mM Tris.HCl, pH 8.0, 89 mM Boric acid, 2 mM EDTA (TBE). The sample buffer consists of 10 mM NaOH, 90% formamide, 1 mM EDTA, containing trace amount of Xylene Cyanol FF and Bromophenol Blue tracking dyes. Gels are run on a Hoefer SE 600 electrophoresis unit at 55 ˚C (31 V/cm, constant voltage). Radioactive gels (e.g., Figure 3a) are quantitated using a Bio-Rad GS-250 Molecular Imager. Non-radioactive gels (e.g., Figure 3b) are quantitated using a Kodak Gel-Logic 200 Imaging System. Care was taken to make sure that no saturation interfered with band quantitation.

Ligation and Analysis.

The solution was brought to 1 mM in ATP and 10 units of T4 polynucleotide ligase (USB) were added. The ligation proceeded at 16 °C for 16 hours. Following ligation, the solution was heated at 90 °C for 5 minutes, and the ligation products were purified using 10% denaturing PAGE. The ligation products were sequenced to establish the correct assembly. A few missed or unknown bases are noted in the experimental sequencing, but these are far from the ligation points, and likely represent errors in the sequencing procedure.

Radioactive Labeling.

Two pmol of an individual strand of DNA was dissolved in 10 μL of a solution containing 50 mM Tris·HCl, pH 7.6, 20 μM spermidine, 10 mM $MgCl_2$, 15 mM dithiothreitol (DTT), and 0.2 mg/mL nuclease free bovine serum albumin (BSA) (US Biochemical) and mixed with 1 L of 1.25 mM γ-^{32}P-ATP (10 μCi/μL) and 3 units of T4 polynucleotide kinase (USB) for 2 h at 37 °C. DNA was recovered by ethanol precipitation.

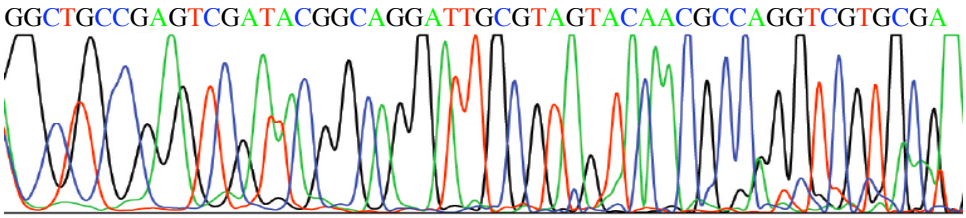
REFERENCES

1. Seeman, N.C., *J. Theor. Biol.* **1982** *99*, 237-247.
2. Seeman, N.C., *J. Biomol. Str. & Dyns.* **1990** *8*, 573-581.
3. Caruthers, M.H., *Science* **1985***, 230*, 281-285.

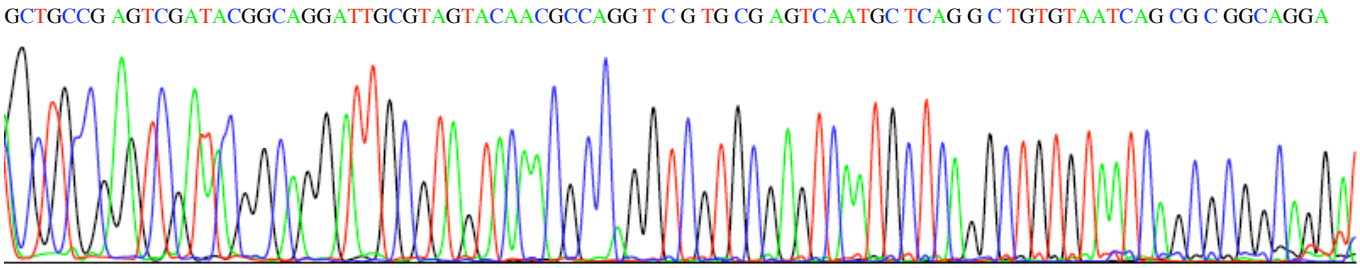
SEQUENCES OF PRODUCT STRANDS

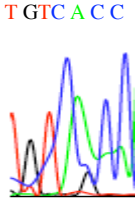
1. Sequencing result of template ABs (Template ABs consists of the ligation (5' --> 3') of strands DA04S and DB08S, S stands for short). The sequence is the complement to the product strand in Figure 2b. It

begins with the leftmost arm of strand DB08S (left of the junction formed by DB07 and DB05), and continues through to strand DA04S.



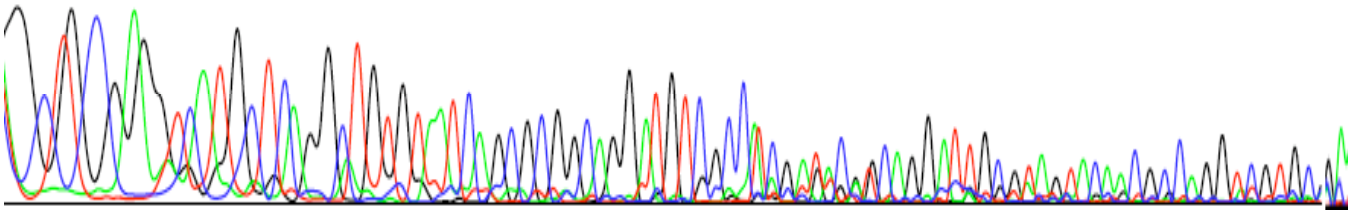
2. Sequencing result of template CAB (Template CAB consists of the ligation (5' --> 3') of strands DC05S, DA04S and DB08S. The sequence is the complement to the product strand in Figure 2c. It begins one nucleotide to the left of the crossover junction involving DB07 and DB05; it continues through to the leftmost DX molecule.





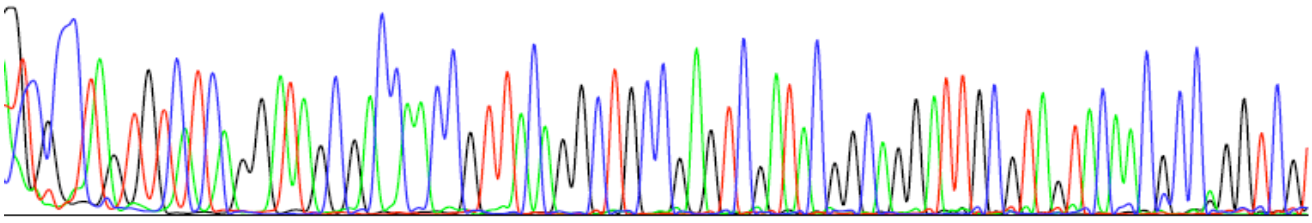
3. Sequencing result of template ACB (Template ACB consists of the ligation (5' --> 3') of strands DA04S, DC05S and DB08S. The sequence is the complement to the product strand in Figure 2d. It begins one nucleotide to the left of the crossover junction involving DB07 and DB05; it continues through to the leftmost DX molecule. There are some sequencing errors in the portion complementary to DA02, but the identity of strand DA04S is not in doubt.

GCTGCCGAGGTCAATGCTCAGGCTGTGTAATCAGCGCGGCAGGATGTCGCCACGATACAGCAGGATTGCGTAGTACAACGCCAGGTCGTGCGA



5. Sequencing result of template ABC (Template ACB consists of the ligation (5' --> 3') of strands DA04S, DC08S and DB05S. The sequence is the complement to the product strand in Figure 2e. The sequence begins four nucleotides to the left of the crossover formed by DC03 and DC01, and continues to through the complements to DB08S and then to DA04S.

GTCGCCTAGTGTCATCAGGATAGCGACCAACCGTTACAGGCTG CCGAGT CGATACGG CAGGATT GC GT AGTA CAA CGCCAGGTCG



T GC GA