Vernier Assembly: Controlling DNA Polymerization via Length Mismatching

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Supporting Information

Material and Methods

Oligonucleotides. All DNA strands were purchased from the IDT and purified by 10-20% denaturing PAGE. The sequence of each strands are listed below:

A2: 5'-$(GCTGTGTATC)_2$-3';
A3: 5'-$(GCTGTGTATC)_3$-3';
A4: 5'-$(GCTGTGTATC)_4$-3';
A5: 5'-$(GCTGTGTATC)_5$-3';
A6: 5'-$(GCTGTGTATC)_6$-3';
A7: 5'-$(GCTGTGTATC)_7$-3';
A8: 5'-$(GCTGTGTATC)_8$-3';
B3: 5'-$(GATACACAGC)_3$-3';
B4: 5'-$(GATACACAGC)_4$-3';
B5: 5'-$(GATACACAGC)_5$-3';
B6: 5'-$(GATACACAGC)_6$-3';
B7: 5'-$(GATACACAGC)_7$-3';
B9: 5'-$(GATACACAGC)_9$-3'.

Formation of DNA Vernier structures. DNA strands (final concentration of total DNA: 0.10 μg/μL) were mixed according to the indicated ratio in a Tris-acetic-EDTA-Mg^{2+} (TAE/Mg^{2+}) buffer. (40 mM Tris base, 20 mM acetic acid, 2 mM EDTA and 12.5 mM magnesium acetate). For Vernier assembly, the sample solutions were first heated to 95 °C for 5 min, then stayed at 65 °C, 50 °C, 37 °C and 22 °C for 2 hrs. at each temperature. The sample solutions were put at 4 °C for overnight before gel electrophoresis.

Native PAGE. Native PAGE containing 6% polyacrylamide (19:1 acrylamide/bisacrylamide) was run on a SE600 cooled vertical electrophoresis unit (Hoefer, 250 V, constant voltage) at 4°C in TAE/Mg^{2+} buffer. After electrophoresis, the gels were stained with Stain-all dye (Sigma), distained by light and scanned by an office HP scanner.

Ligation. A designated DNA strand (30 μg in 30 μL) was first phosphorylated by 5 units of T4 polynucleotide kinase (New England Biolabs) at 22 °C for 2 hours in the commercial kinase reaction buffer (70 mM Tris-HCl, 10 mM MgCl2, 5 mM DTT, 1mM ATP, pH 7.6) supplemented with 1.0 mM ATP, followed by incubating at 95 °C for 5 min to inactivating the kinase. Then the kination mixture (including the phophorylated strand) was mixed with the other unphosphorylated strand (30 μg in 30 μL the same buffer mentioned above) according to the designated ratio and annealed from 65 °C to 22 °C over 3 h. Finally, 10 units of T4 DNA Ligase (New England Biolabs) were added and the ligation was performed by incubating the solution at 22 °C for ~ 16 hrs. Then the DNA sample was analyzed by 12% denaturing gel (19:1 acrylamide/bisacrylamide, containing 8.3 M urea) at 55 °C.

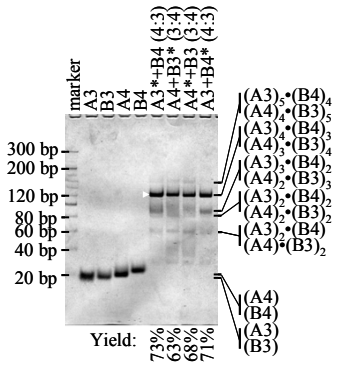

Figure S1. Native polyacrylamide gel electrophoresis (PAGE) analysis of Vernier assembly of 120-bp-long DNA duplexes at 22 °C. The sample composition of each lane, chemical identity of each band, and the assembly yields are indicated above, beside, and below the gel images, respectively. * indicates that the strand is phosphorated at 5' end. White arrowheads indicate the desired product positions. Note that for each Venier assembly, two independently, parallel samples are prepared by cooling the DNA solutions from 95 °C to 22 °C. Assembly yield varies in a small range, suggesting that the Vernier assembly is reproducible.