Supplementary Material for Chemical Communications

Template-Free, Polymerase-Free DNA Polymerization

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Supporting Information

Experimental Section

DNA Oligonucleotides. (a) DNA sequences were designed by a computer software, named "SEQUIN".[1] Primer: 5'-gCA TAC TgT CgT TCC TTT Tgg AAC gAC AgT ATg Cgg C-3'; Monomer: 5'-CAg gCC ggC TAA ATg ATT ATT TTT AAT CAT TTA gCC ggC CTg gCC-3'. All oligonucleotides were purchased from Integrated DNA Technologies, Inc., and purified by denaturing polyacrylamide gel electrophoresis.

Radioactive isotope labeling. A 20 μl mixture solution of 1 pmole primer strand, 1 μl of 2.2 μM [γ-^{32}P]ATP (10 mCi/mL), 3 units of polynucleotide T4 kinase (New England Biolabs, Inc., NEB) in a kination buffer, which contained 66 mM Tris-HCl, pH7.6, 6.6 mM $MgCl_2$, and 10 mM dithiothreitol (DTT), was incubated for 2 hrs at 37˚C. The reaction was stopped by heating the reaction mixture at 90˚C for 5 minutes. Then the labeled DNA strand was purified by 20% denaturing polyacrylamide gel electrophoresis.

Denaturing Polyacrylamide Gel Electrophoresis (PAGE). Gels contained 20% polyacrylamide (19:1 acrylamide/bisacrylamide) and 8.3 M urea; they were run at 55˚C. The running buffer is a Tris-Borate-EDTA buffer (TBE), which consisted of 89 mM Tris buffer (pH 8.0), 89 mM boric acid, and 2 mM ethylene diamine tetraacetic acid (EDTA). Gels were run on a Hoefer SE 600 electrophoresis unit at 600 V (constant voltage).

Native PAGE. Gels contained 8% polyacrylamide (19:1 acrylamide/bisacrylamide) and were run on a FB-VE10-1 electrophoresis unit (Fisher Biotech) at 22˚C (80 V, constant voltage). After electrophoresis, the gels were exposed and scanned by a PhosporImager (Packard).. TBE buffer were used for electrophoresis.

DNA polymerization. Primer and monomer strands at designated ratios were dissolved in 30 μl NEB Buffer 3 (containing 100 mM NaCl, 50 mM Tris-HCl, pH 7.9, 10 mM $MgCl_2$, 1mM DTT), to which the ATP concentration was brought to 1 mM. The final primer concentration is 0.1 μM. Polymerization is initiated by addition of 1,200 units of T4 polynucleotide ligase (NEB) and 30 units of Bgl I (NEB). At different incubation time, an aliquot of 3 μL of the reaction mixture was withdrawn, mixed with 7 μL stopping buffer (20 mM EDTA in formamide), and quenched to dry ice, and then analyzed by native PAGE.

Reference

1. Seeman, N. C. *J. Biomol. Struct. Dyn.* **1990**, *8*, 573.