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Supplementary Material

A Highly Convenient Procedure for Oligodeoxynucleotide Purification

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RECIPES FOR PREPARATION OF POLYMERIZATION SOLUTIONS

3.7 M of *N*,*N*-Dimethylacrylamide and 0.37 M *N*,*N*′-methylenebis(acrylamide) solution (cross-linking ratio 10:1): To 103 µl *N*,*N*-dimethylacrylamide (MW 99.13 mg/mmol, density 962 µg/µl, 100 mg, 1 mmol) and 15.5 mg *N*,*N*′-methylenebis(acrylamide) (MW 154.17 mg/mmol, 0.1 mmol), add 103 µl water. Mix to dissolve. The final volume of the solutions is 272 µl.

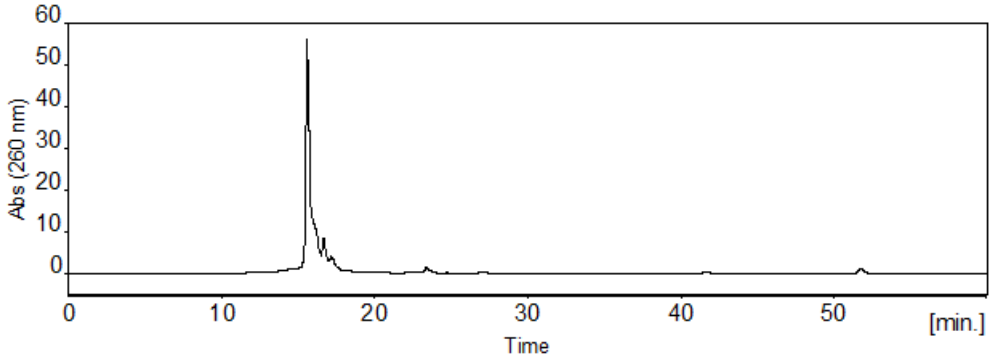
5% $(NH_4)_2S_2O_8$ Solution: To 5 mg $(NH_4)_2S_2O_8$ (MW 228.18 mg/mmol), add 95 µl water. Mix well (5 µl solution contains 1.1 µmol $(NH_4)_2S_2O_8$).

0.66 M TMEDA solution: To 49 µl TMEDA (MW 116.24 mg/mmol, density 0.777 mg/µl, 38.1 mg, 0.33 mmol), add water until the total volume reaches 500 µl. Mix well (5 µl solution contains 3.3 µmol TMEDA).

HPLC Conditions

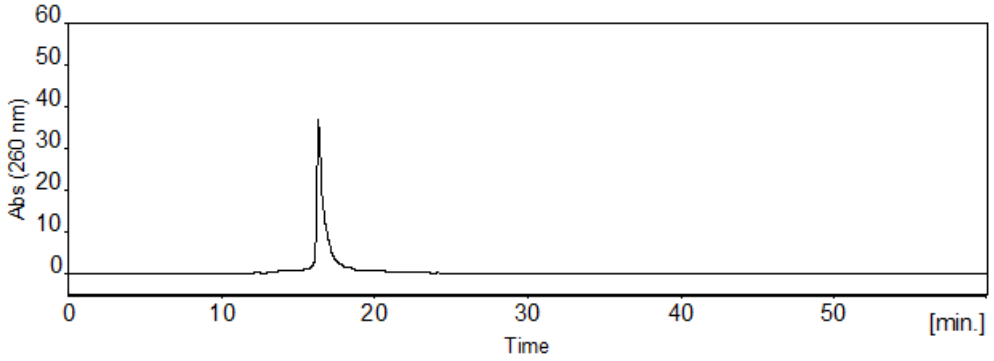
Column: C-18, 5 µm, 100 Å, 250 × 3.20 mm; solvent A: 0.1 M triethylammonium acetate, 5% acetonitrile; solvent B: 90% acetonitrile; flow rate: 0.5 mL/min; detection: 260 nm; faster gradient: solvent B (0%-45%) in solvent A over 60 min; slower gradient: solvent B (0%-15%) in solvent A over 60 min.

HPLC of Crude ODN 2, Faster Gradient



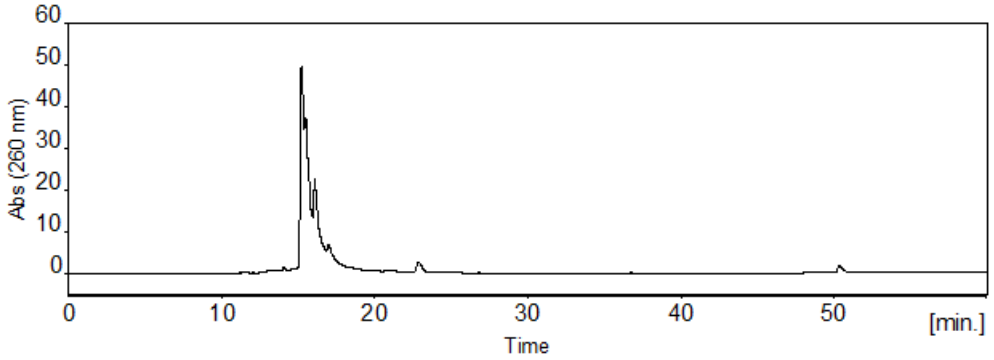
The enlarged version of the profile shown in trace a in Figure 2. Failure sequences were capped with polymerizable phosphoramidite during synthesis, and thus have longer retention times than the full-length sequence. ODN **2** is 5′-TCA TTG CTG CTT AGA CCG CT-3′.

HPLC of Pure ODN 2, Faster Gradient



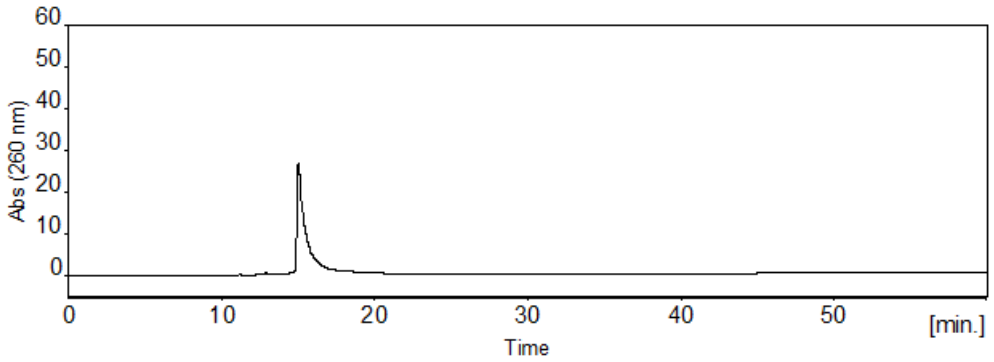
The enlarged version of the profile shown in trace b in Figure 2. The ODN was purified by catching failure sequences by polymerization.

HPLC of Crude 19-mer ODN 3, Faster Gradient



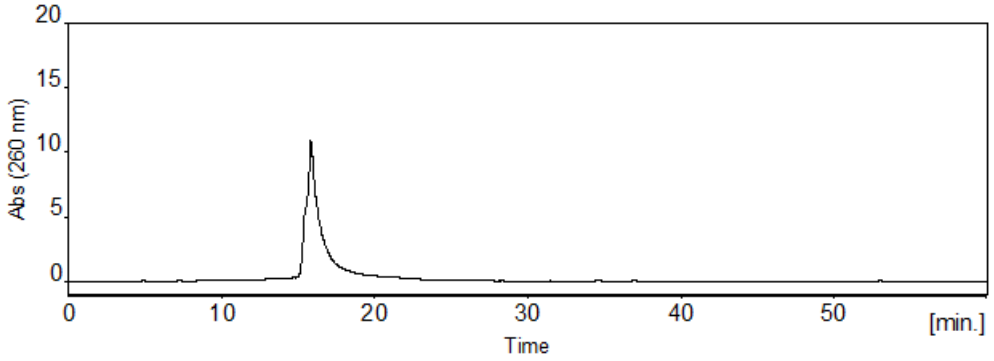
The 19-mer ODN **3** (5′-CAT TGC TGC TTA GAC CGC T-3′), which is the same as **2** except that there is one less base at the 5′-end, was synthesized using the same procedure for **2**.

HPLC of Pure 19-mer ODN 3, Faster Gradient



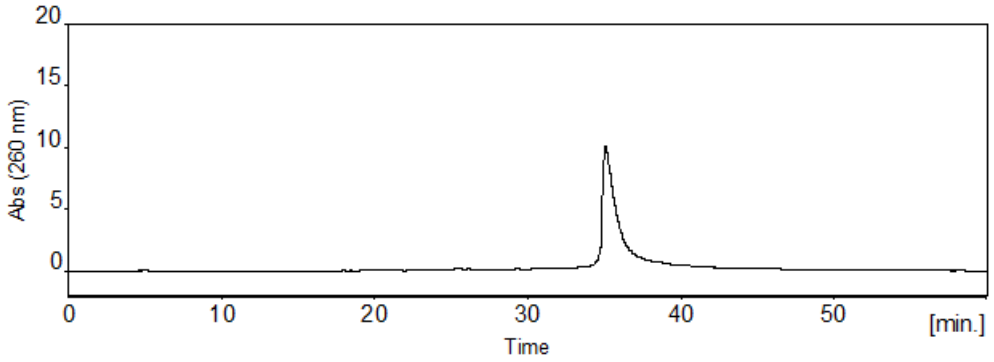
The 19-mer ODN **3** (5′-CAT TGC TGC TTA GAC CGC T-3′) was purified using the same procedure for **2**.

HPLC of ODNs 2 and 3, Faster Gradient

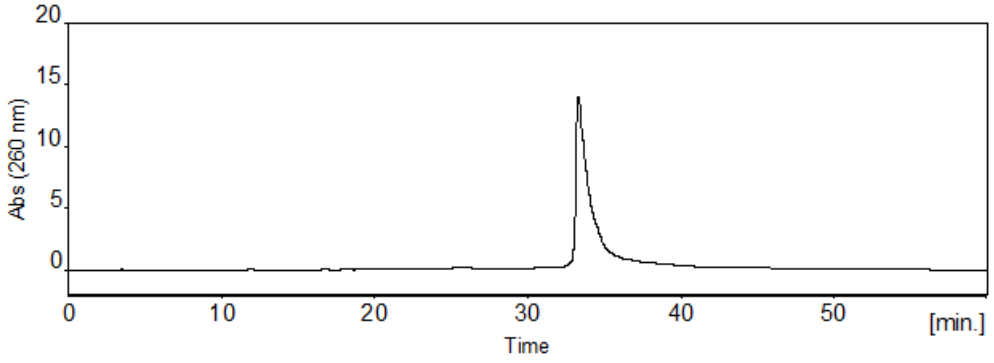


ODN **2** and **3** were co-injected into HPLC. A broadened peak was observed indicating that HPLC can resolve the 19-mer failure sequence from 20-mer full-length sequence.

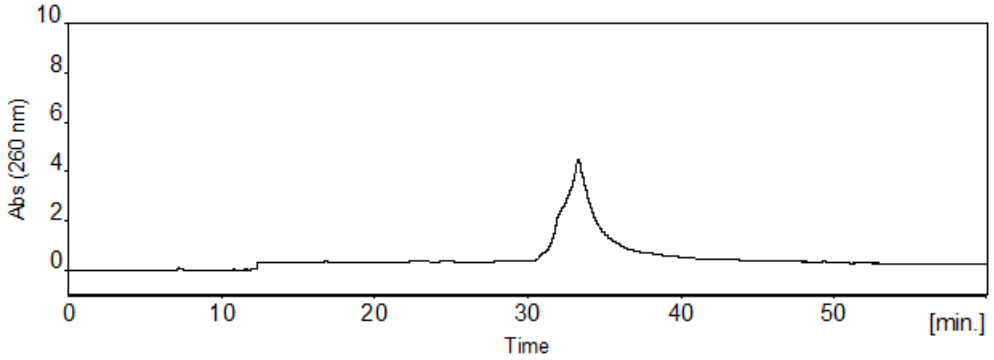
HPLC of Pure ODN 2, Slower Gradient



HPLC of Pure 19-mer ODN 3, Slower Gradient

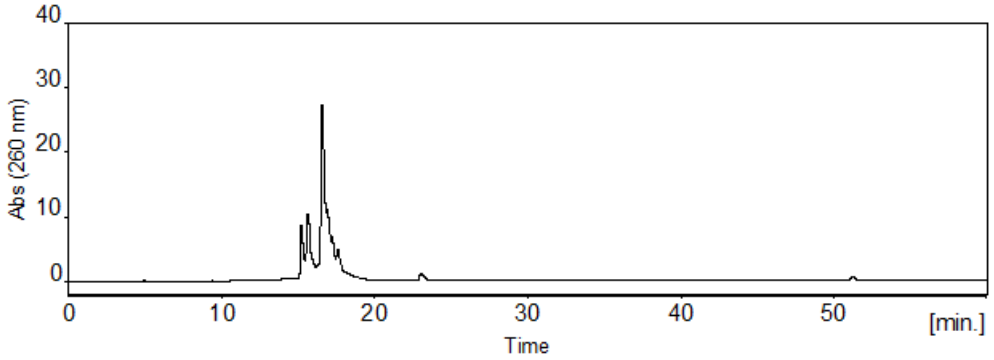


HPLC of ODNs 2 and 3, Slower Gradient



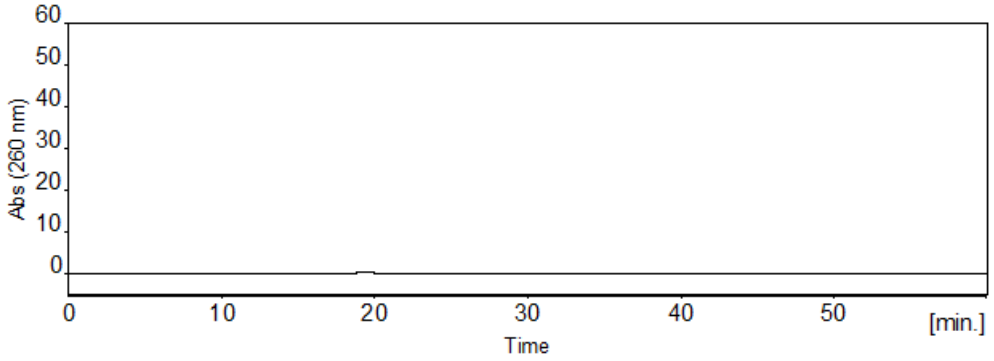
ODNs **2** and **3** (~1:1 ratio) were co-injected into HPLC. With slower gradient, the two were better resolved. Under the same conditions, pure ODN **2** does not show a shoulder at the left side of the peak.

HPLC of 20-mer ODN 4 Containing 8-oxo-dG Treated with Ammonia



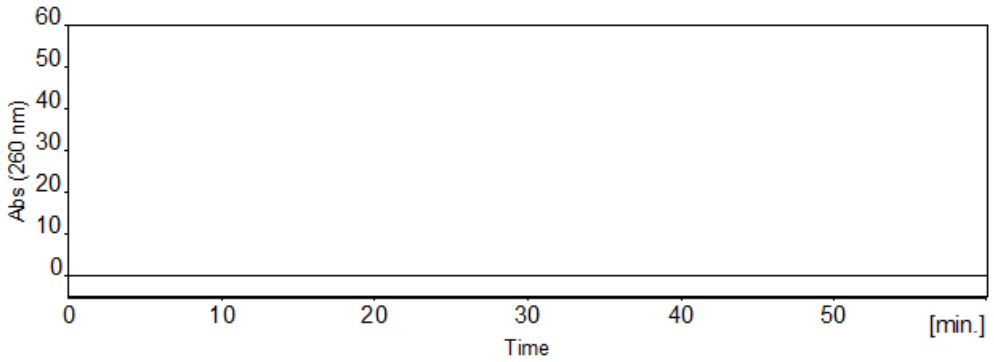
The 20-mer ODN (**4**) that contains an 8-oxo-dG [5′-TCA TTG CT(8-oxo-dG) CTT AGA CCG CT-3′] was synthesized and purified with reversed-phase HPLC (*RSC Adv.* **2014**, *4*, 8746). The product was heated in concentrated NH_4OH at 80 °C for 15 min. After cooling to room temperature, *n*BuOH was added. The precipitate was analyzed with HPLC. The fast gradient was used. The multiple peaks in the profile indicate that the ODN is not stable under such conditions. Because ODN **2** was also processed under the same conditions during purification and only showed a single peak, we infer that **2** does not contain damaged dG. This experiment gives additional proof that ODN is stable under radical acrylamide polymerization conditions.

HPLC of Residue from 4th Extraction of Gel



During purification of ODN **2** using the catching by polymerization approach, after extraction of full-length sequence with water for three times, the gel was extracted for an another time. The 4th extract was processed using the same *n*BuOH precipitation procedure and analyzed with HPLC. No UV active material is detectable indicating that three extractions are sufficient.

HPLC of blank control experiment



The catching failure sequences by polymerization ODN purification procedure was followed except that crude ODN was not added. After *n*BuOH precipitation and removal of supernatant, no solid residue was observable in the tube. The tube was washed with 30 µl water, and 20 µl was injected into HPLC to generate the profile, which showed no UV active material.