Supplementary Material

A Highly Convenient Procedure for Oligodeoxynucleotide Purification

Durga Pokharel and Shiyue Fang

Department of Chemistry, Michigan Technological University, 1400 Townsend Drive, Houghton, MI 49931 USA

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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% (NH₄)₂S₂O₈ Solution: To 5 mg (NH₄)₂S₂O₈ (MW 228.18 mg/mmol), add 95 µl water. Mix well (5 µl solution contains 1.1 µmol (NH₄)₂S₂O₈).

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 x 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

![Graph of HPLC profile of Crude ODN 2](image)

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

![Graph of HPLC profile of Pure ODN 2](image)

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

![Graph of HPLC profile of Crude 19-mer ODN 3](image)

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 C(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 NH₄OH at 80 °C for 15 min. After cooling to room temperature, nBuOH 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 another time. The 4th extract was processed using the same nBuOH 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 \textit{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.

![HPLC profile](image)

The graph shows the UV absorbance at 250 nm over time. No active material was detected throughout the 50-minute period.