High performance DNA oligonucleotide purification using Agilent TOP-DNA

Application Note

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Introduction

Because of the significant progress achieved in the solid phase chemical synthesis of oligonucleotides during the past four decades, synthetic oligonucleotides have become readily available and have fuelled the biotechnology revolution that has irreversibly changed biomedical research and the pharmaceutical industry.

Although a powerful technique, solid phase synthesis has some drawbacks. The main limitation is the need for very high coupling yields in every chain extension step. A consequence of yields of <100% is the accumulation of failure sequences containing deletions. Separation of the full-length product from the shorter failure sequences and especially the n-1 failure sequence is a significant problem. This purification step becomes more difficult as the oligonucleotide length increases.

Trityl-on purification enhances the selectivity between the full length sequence and the truncated failures. Solid phase extraction (SPE) tubes provide a low cost method for this type of purification. Historically, these are capable of performing efficient separations for oligos up to 50 bases in length. Yields for longer chain oligomers tend to be rather poor and the technique generally not applicable.

Agilent has developed an optimized tube system, trityl-on oligonucleotide purification (TOP), specifically designed to work for DNA oligomers up to 150 bases. The method is applicable to the ammonia and methylamine deprotection strategy and can purify as few as one, or as many as 96 DNA oligonucleotides in 10 to 15 minutes using a vacuum procedure (requires the Agilent VersaPlate baseplate, part no. 75700001).

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Materials and reagents

- TOP-DNA 150 mg tubes, 96/pk - part no. 7572915C
- Vac Elut 20 manifold - part no. 12234100
- Acetonitrile (HPLC grade)
- Trifluoroacetic acid (99%)
- 2 M triethylammonium acetate (TEAA)
- 5% trifluoroacetic acid in deionized water
- 50:50 acetonitrile:water
- NaCl solution (100 mg/mL)

Oligonucleotide

The oligonucleotide should be cleaved from the synthesis support and base deprotected using standard procedures (typically concentrated ammonia cleavage and ammonia and methylamine base deprotection). The oligos in either the ammonia or amine solution are then ready for purification following the method below.

Recommended loadings for a 150 mg TOP-DNA tube will vary according to the oligomer chain length.

Guidelines for the maximum:

≤ 40mer	1 µmol scale
≤ 75mer	500 nmol scale
> 100mer	200 nmol scale

Method

1. **Sample treatment.** Add NaCl solution (100 mg/mL) 1:1 v/v ratio to each sample to be purified.
2. **Condition tube.** With the vacuum on (7 in Hg), add 0.5 mL of acetonitrile to the tubes. A drop rate of approximately 1 to 2 drop/sec would be anticipated. The vacuum may remain on and unadjusted through step 8.
3. **Equilibrate tube.** As soon as possible add 1 mL of 2 M TEAA to the tubes.
4. **Apply sample.** Add pretreated sample. The sample can be added in 1 mL aliquots.
5. **Wash.** Add 2 x 1 mL of NaCl solution (100 mg/mL).
6. **Detritylate.** Add 2 x 1 mL of 5% trifluoroacetic acid in deionized water.
7. **Rinse.** Add 2 x 1 mL of water.
8. **Prepare to collect sample.** Remove the cover, using the vacuum release valve. Place a collection tube in the correct position in the rack and replace the cover.
9. **Elute.** Add 1 mL of 50:50 acetonitrile:water.

The purification should take approximately 10 to 15 minutes if carried out without any breaks between the additions of the solutions to the tube.

Results

A series of trityl-on DNA oligomer sequences were purified using the above protocol on 150 mg TOP-DNA tubes. A summary of the yield and purity data for the purified products is provided below in Table 1, and HPLC chromatographs in Figures 1, 2 and 3.

Table 1. *Purity and yield values from reversed-phase chromatograms*

DNA length	Sequence	Purity	Yield
21mer	Figure 1	92%	92%
70mer	Figure 2	96%	93%
120mer	Figure 3	90%	97%

HPLC Conditions

HPLC analysis of crude and TOP-DNA tube-purified DNA.

Column:	Agilent PLRP-S 5 µm, 4.6 x 150 mm (part no. PL1111-3500)
Mobile phase A:	100 mM TEAA, pH 7
Mobile phase B:	Acetonitrile
Gradient:	A/B (95:20) from 0 to 20 minutes
Flow rate:	1 mL/min
Detection:	UV @ 256 nm, temperature ambient

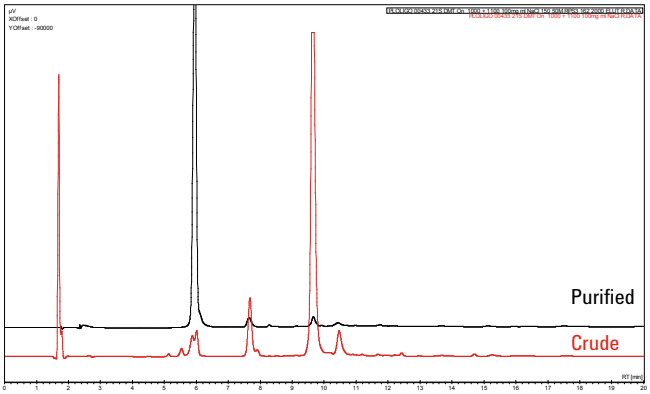

Figure 1. *Purification of a 21mer DNA sequence - 5′(DMT) GAC TGA ATG GCT GAT CTA CGT 3′*

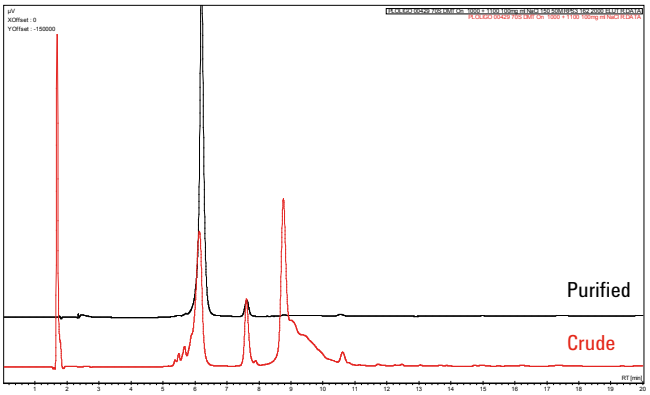

Figure 2. *Purification of a 70mer DNA sequence - 5′(DMT) GAC TGA ATG GCT GAT CTA CGT ATC CTA ATC GCG ATC CTA GTC ACG GTC CAT CTG GCT TAA CGT CGA AAC 3′*

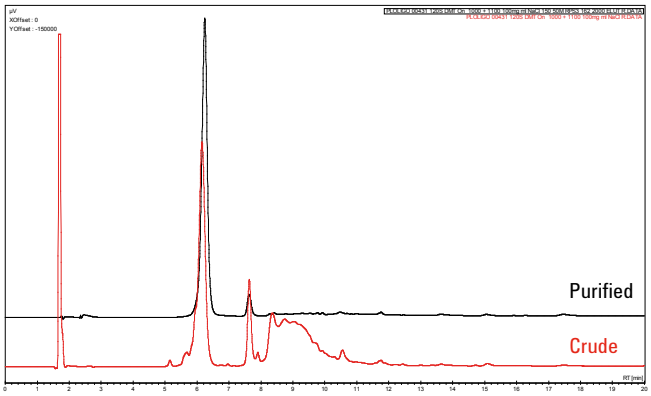

Figure 3. *Purification of a 120mer DNA sequence - 5′(DMT) GAC TGA ATG GCT GAT CTA GCT ATC CTA ATC GCG ATC CTA GTC ACG GTC CAT CTG GCT TAA CGT CGA AAC GAC TGA ATG GCT GAT CTA GCT ATG CTA ATC GCG ATC CTA GTC ACG GTC CAT 3′*

An example of a crude and purified polyT 100mer using the above protocol analyzed using CE is shown in Figures 4 and 5.

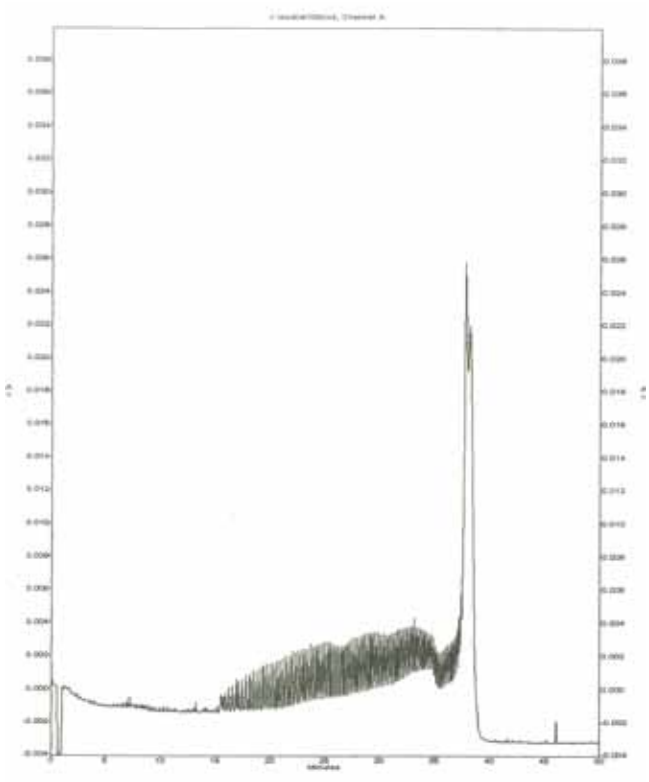

Figure 4. *Crude 100T*

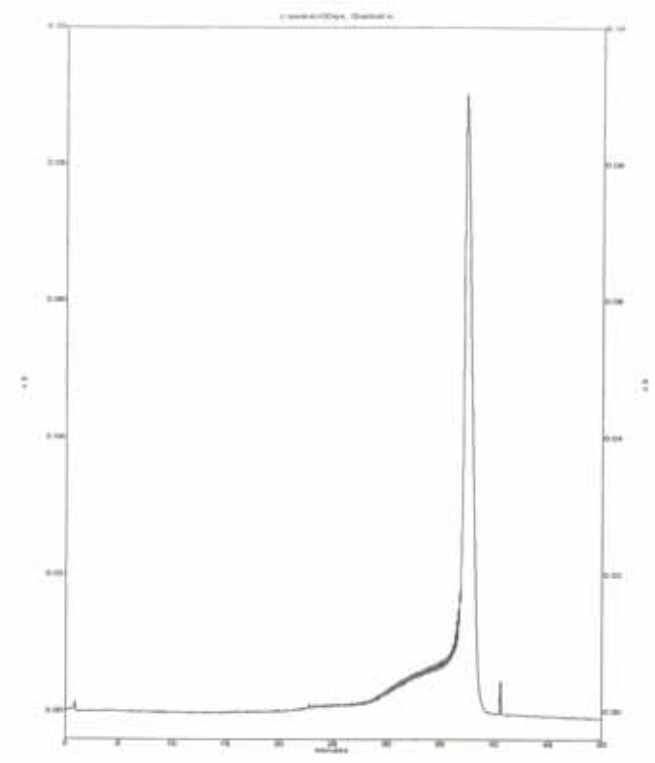

Figure 5. *Purified 100T*

Comparative study

Comparison of yield data for a polyT 100mer using various alternative tubes and recommended protocols highlights the enhanced performance of the Agilent TOP-DNA system. Results are shown in Table 2.

Table 2. *Comparative yields from alternative tubes*

Cartridge	Yield (reversed-phase data)
Alternative I	44%
Alternative II	57%
Alternative III	25%
TOP-DNA	96%

Conclusion

This protocol, combined with the TOP-DNA high-affinity sorbent, 96-well format and optimized reagents, provides a simple, high-throughput process for effective purification of both long and short chain DNA oligonucleotides, providing both high yields (> 85%) and high purity (> 90%).

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