Large Scale Purification of SBS Oligos using HPLC (10 μmole scale)

Purpose: To purify the SBS oligos from failed sequences, remove the DMT group, and remove all salts for NMR use.

General Advisories:

1. The oligos are synthesized with a DMT group on the last nucleotide, which is critical for the first purification step using the C4 column on the HPLC. This group is rather unstable, and the oligos should be protected from light and processed right away upon receipt.
2. The oligos should never be stored in acidic conditions – this will cause removal of the DMT tag and/or depurination of the nucleotides
3. Work with each oligo separately. The oligos should never be combined until the sequences and purity are confirmed with the proton NMR spectrum

Materials Needed:

SBS oligos:

Sense – 5'- GGC TCA GGG CCA CAG –3'
Anti – 5'- CTG TGG CCC TGA GCC – 3'

Buffers:

1. HPLC Buffer A: 0.1 M Triethyl ammonium acetate (TEAA) pH 6.5
   (for 2L: 27 mL Triethylamine, 12 mL acetic acid)
2. HPLC Buffer B: 80% Acetoniltrile
3. 80% Acetic acid/H2O
4. SF-1 DBD NMR Buffer: 20 mM d-Tris acetate, 2 mM d-DTT, 50 μM $ZnCl_2$, 0.2% Sodium Azide, 10% D_2O pH 6.0)

Procedure:

A. Separate complete sequences (with DMT group) from failed sequences using a C4 column on the HPLC

Work with each oligo separately. The DMT group is relatively unstable, so this step should be performed for the sense and antisense strands right away upon receipt.

1. Oligos purchased from TriLink Biotechnologies will be received as a frozen 2 mL suspension
2. Thaw the oligos and measure the UV spectrum (2 μL into 500 μL should be a sufficient dilution)
3. Calculate the concentration by dividing the absorbance at 260 by the extinction coefficient
4. Add 18 mL of 0.1 M TEAA (pH 6.5), bringing the volume up to 20 mL
5. Perform a scouting run on the HPLC by injecting 2-3 mL.
   Starting conditions: 95% Buffer A, 5% Buffer B, with Buffer B increasing at a rate of 2% per minute) There should be two major peaks: one at approx 25% B (failed sequences) and a second peak at approx 40-45% B (complete sequences + DMT)

6. Once the run conditions are determined, the starting conditions can be changed: 85% Buffer A, 15% Buffer B with Buffer B increasing at a rate of 2% per minute. The injection volume can also be increased.
7. Between injections, the column must be washed for 10 min with 95% Buffer B, and subsequently equilibrated with the starting buffer conditions for 10 minutes
8. Lyophilize all of the peaks collected at 40-45% B
9. Resuspend and combine the antisense collection tubes in 5-10 mL of UV/UF water and measure the OD260 to determine approximate yield.
10. Repeat #9 for the sense strand
11. Freeze the oligos first at –20 °C and then at –86 °C and lyophilize the oligos again for the next step

B. Remove the DMT group from the purified oligos and Isolate the oligos from the DMT group using HPLC

1. Using a 50 mL tube, dissolve the tritylated oligos in 5 mL of 80% acetic acid
2. Vortex well (a reminder: always make sure the cap is on securely on)
3. Let stand for 20 minutes at room temperature
   Note: the solution should turn orange
4. Add ~ 15 mL of 0.1M TEAA (buffer A) to neutralize the pH of the sample
5. **IMMEDIATELY** purify the oligos with reversed phase HPLC (95% Buffer A and 5% Buffer B). The oligo should elute at approximately 20-25% B.
6. Check for a second peak around 40% B, which corresponds to oligos still retaining the DMT group.
7. Lyophilize the peaks collected at 20-25% B

C. Remove salt (triethylammonium) from each oligo with HiPrep Desalting 26/10 and Dowex ion exchange columns

1. Charge the Dowex resin column (12 mL) with ~150 mL of 2 M LiCl using the BioRad Econo pump
2. Connect the HiPrep Desalting column to the Akta Purifier and wash the column to remove the 20% ethanol storage buffer
3. Once the Dowex column is charged, connect the Dowex column to the Akta Purifier in tandem <u>after</u> the HiPrep column.
4. Wash both columns extensively with filtered UV/UF water until the conductance reading is zero
5. Connect the 5 mL superloop to the Akta Purifier and wash extensively with UV/UF water
6. Resuspend the oligos in ~10 mL of filtered UV/UF water. KEEP ON ICE
7. Perform a scouting run with 2 mL of oligos. Do not load more than 4 mL in the 5 mL injection loop.
8. Run the method “Hiprepdnadesalting” in Tanya’s directory (isocratic elution with Buffer A: filtered UV/UF water, Flow rate: 5 mL/min)
9. Perform manual fraction collection. There should be a clear separation between the DNA peak and the conductance peak (otherwise load less sample). The DNA peak elutes around 17 mL to 27 mL
10. Lyophilize the collected DNA

11. After every run, the column that is packed with dowex resin should be regenerated with ~12 mL 2 M LiCl. This is done by removing the top connector and adding the salt solution.

D. NMR Sample preparation and Duplex formation

1. Resuspend the purified oligos in 500 µL of 99.996% D_2O (pH to 6.0)
2. Measure the OD260 to determine the concentration of each strand
3. Perform 1D proton NMR analysis on each strand separately
4. If the oligos are the correct composition, create the **DNA duplex**: add an equimolar amount of sense and antisense strands to an Eppendorf tube, heat to 65 °C for 5 min and cool on ice.
5. Run a 1D proton and 2D TOCSY on the duplex in 100% D_2O
6. Lyophilize the duplex, resuspend in 10% D2O and record the 1D proton spectrum to characterize the imino proton pattern.