Oligonucleotide Purification

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Purpose: Purification of oligonucleotides may be necessary for very long oligonucleotides, depending on the downstream application.

1. Use PAGE to purify 20-30 micrograms of oligonucleotide on a small 10% denaturing acrylamide urea gel (1 mm thick small gel). To do this, mix equal volumes of oligonucleotide and formamide gel loading buffer (max volume for our gels is 40 microliters, but 20 microliters total is a preferred volume). Heat at 100 degrees 1-2 min and quick chill on ice. Load sample to gel which has been pre-run for 15-20 min at 240 V. Clean urea out of gel wells before loading sample. Run gel until desired separation is obtained (typically until bromophenyl blue is about 3/4 of the way to the bottom; ~1.5 hrs for a 10% gel).

2. Visualize the oligonucleotides using UV shadowing. Place the gel on saran wrap and place on top of an intensifying screen. Shining short wave UV light from a hand held lamp will reveal the oligonucleotide. Be careful not to expose the DNA to UV light any more than absolutely necessary and wear appropriate eye and face protection.

3. Excise the desired gel slice and transfer to a 5 ml syringe which has been placed in a 15 ml screwcap tube. Crush the gel slice with the syringe plunger. Carefully remove the plunger and rinse with 3 ml of TE. Remove the syringe and incubate the crushed gel slice/TE on the tube roller at 37 degrees for ~ 4 hr or overnight.

4. Dump the crushed gel slice solution to a Fisher screening column which has been placed in a 15 ml conical tube. Centrifuge for 1-2 min in clinical centrifuge. This step removes large pieces of acrylamide.

5. Add the equivalent of 0.3 ml packed DEAE sepharose FF resin to an empty BioRad spin column. Add 1.5 ml TE. Place column in 13 x 100 mm glass test tube. Spin in clinical centrifuge (12-place fixed angle rotor) on setting 2 for ~1 min until all liquid is out of the resin (don't over centrifuge as this may damage the resin).

6. Add 1.5 ml oligo sample from step 4 and spin ~1 min on setting # 2. Repeat until all sample loaded.

7. Wash with 1-1.5 ml of 0.1 M NaCl, 50 mM Na Acetate, pH 5.5.

8. Transfer column to a plastic Sarstedt tube (75 x 12 mm). Add 0.8 ml 1.0 M NaCl, 50 mM Na Acetate, pH 5.5. Spin in clinical centrifuge on setting 1 for ~2 min.

9. Remove liquid containing the oligonucleotide and transfer 0.4 ml to each of two eppendorf tubes. Fill to the top with ETOH and precipitate.

If a number of oligonucleotides are being purified at the same time, it is very easy to cross contaminate the oligos at the ETOH precipitation step as the pellets are very small and unstable. Use a different disposable tip to remove the supernatant from each oligo pellet to minimize contamination between oligos.

Wash with ~300 microliters of 100% ETOH. Dry and resuspend in 10 microliters H2O total volume. Measure recovery by absorbance.

Typical recovery is ~15-20% of starting material.