ISOLATION AND ANALYSIS  OF BAY SCALLOP ABDUCTOR MUSCLE DNA

A.  **Introduction:** In this procedure you will isolate DNA from bay scallop abductor muscle by a method that avoids the use of phenol/chloroform (CHCl3) extraction. This is the same method that you used in Biochemistry 1 with bovine thymus gland tissue. After the isolation of the DNA you will then use an UV spectrophotometric method to determine the concentration of your isolated bay scallop DNA solution. This is required so that you can calculate how much DNA is required for endorestriction nuclease digestions later. In addition, you will calculate the $A_{260}/A_{280}$ ratio to evaluate the purity of your group’s DNA preparation. To refresh your memory on these techniques please re-read the Visible, UV and Mass Spectrometry section and Experiment 1: “Determination of the Identity and Concentration of a Complex Biological Mixture”.

**Isolation:** DNA molecules are the largest linear covalent structures found in nature. In some cells these molecules exceed $10^9$ g/mole in molecular weight. Mammalian cells such as lymphocytes and cells from liver and thymus tissues are excellent sources of genomic DNA. Animal cells are easily lysed by a mild detergent such as SDS (Sodium Dodecyl Sulfate), therefore, no lysozyme is needed to open up a cell wall. In this protocol, after the cells are lysed, and proteins are hydrolyzed by proteinase K and RNA is hydrolyzed with the enzyme RNase. Proteins are removed from the DNA by a simple salting out procedure and RNA is trapped in the pellet obtained after centrifugation. DNA (and RNA) is readily soluble in low salt buffers as long as the pH is above 7. However, above approximately 70% ethanol or 35% isopropanol, the nucleic acids precipitate. The conditions of alcohol precipitation are chosen to balance the recovery and yield of nucleic acid against its purity. Colder precipitation temperatures, longer precipitation or centrifugation times, and higher proportions of alcohol lead to higher recoveries at the expense of purity. DNA yields for the following method are similar to that obtained from the phenol/CHCl3 extraction method. Using this method, one can expect 3 - 4 µg
Hydrolysis of DNA by DNases is inhibited by EDTA to chelate and remove Mg\(^{2+}\) ions which are required for the complete activity of DNase. The DNase activity can also be inhibited by using: a buffer with a pH greater than the optimum of DNases (pH > 8.0); the detergent SDS to denature the DNases; a heating step to denature the DNases; and the enzyme proteinase K to hydrolyze DNases and other proteins, such as histones associated with ribosomal RNA.

Analysis by UV Spectroscopy: The absorbance at 260 nm is roughly proportional to the nucleic acid concentration. The total UV absorbance is the sum of the absorbances of the individual nitrogenous bases. The nucleotide spectra is a function of pH and reflects the titration of specific functional groups of the bases. Extensive secondary structure, as found in native duplex DNA reduces the UV absorbance due to stacked hydrogen bonded bases in the interior of the double helix. Thus, a 0.050 mg/mL solution of duplex DNA gives an \(A_{260} = 1.0\) at neutral pH in a 1.0 cm path length cuvette. By knowing the characteristics of the DNA and setting up a series of dilutions of your isolated stock solution of DNA (such that the absorbance values between 0.5 and 1.0 are obtained), the concentration can be determined. The ratio of absorbances at 280 nm and 260 nm can be utilized to estimate the purity of a preparation. A pure solution of double stranded DNA free of RNA and proteins has an \(A_{260}/A_{280}\) ratio = 2.0. Contamination with protein gives an \(A_{260}/A_{280}\) ratio less than 1.8. Additionally, the ratio may be lowered due to contaminating organic solvents. A \(A_{260}/A_{280}\) ratio between 1.8 and 2.0 is considered suitable for PCR, restriction digests, or Southern blots.

II. Materials:

A. 1. Bay scallop abductor muscle tissue, frozen and ground to a powder: \(~500 – 1800\) mg.

   2. Lysis buffer (10 mM Tris-Cl, pH 8.2, 400 mM NaCl, 2 mM Na\(_2\)EDTA): 6.0 mL
3. Proteinase K solution, 2 mg/mL in 1 % (w/v) SDS, 2 mM Na₂EDTA: 1.0 mL.
4. 10 % (w/v) SDS: 0.4 mL.
5. Culture tube.
6. RNase A solution, 4 mg/mL in lysis buffer: 30 µL.
7. Saturated NaCl (~6 M): 2.0 mL.
8. 50 mL beaker.
9. Beckman J2-MC centrifuge, JA-14 rotor, 15.0 mL adapters.
10. 100% and 70% Ethanol: 10 mL and 2 mL.
11. TE buffer (10 mM Tris-Cl, pH 7.5, 1.0 mM Na₂EDTA): 1.0 mL.
13. Filter and sterile glass wool.
15. Water baths at 37 °C and 65° C.
16. Sterile culture tubes.

B. 1. Isolated thymus gland DNA solution.
   2. TE buffer (10 mM Tris-Cl, pH 7.5, 1.0 mM Na₂EDTA).
   4. 2.0 mL microfuge tube.
   5. Spectrophotometer and cuvettes: Beckman DU 640 spectrophotometer and 1.0 cm path length quartz microcuvettes.

III. Methods:

Week 1- Note: your group will have to come back before the next lab to finish Parts A and B)

A. 1. Record the mass of a 15 mL Oak Ridge centrifuge tube, and then add to it the entire amount of frozen, powdered bay scallop tissue. Re-weigh the tube and determine the actual mass mass of the thymus gland.

2. Suspend the tissue in 6.0 mL of lysis buffer. Add 0.4 mL of 10% SDS and 1.0 mL of the Proteinase K solution.
3. Digest sample for 2 hours in a 65°C water bath. Periodically shake contents to enhance the digestion.

4. Transfer the centrifuge tube containing the digested solution to a 37°C water bath and add 30 µL of the RNase A solution. Mix by inverting the tube 25 times. Incubate at 37°C for 15 minutes.

5. Cool the tube to room temperature by running tap water along the outside of the tube. After 5 min. at room temperature, add 2.0 mL of the saturated NaCl solution. This step precipitates proteins. Rapidly invert 200 times to thoroughly mix. Centrifuge at 12,000 x g (9000 rpm with a JA-14 rotor) for 10 minutes at room temperature.

6. Carefully filter the supernatant containing the DNA through a stemless funnel with a glass wool plug into a 50 mL beaker and add 2 volumes (~18.8 mL) of room temperature 100% ethanol. Mix gently until DNA precipitates; allow the solution to sit at room temperature for at least 5 min. (This is a potential stopping point to store samples in refrigerator)

7. Carefully wind the DNA on to a sealed Pasteur pipette. If there is no DNA to wind, place the DNA solution on ice for 15.0 min. Centrifuge at 12,000 x g (9000 rpm) with a JA-14 rotor. Carefully pour off the supernatant.

8. Wash the collected DNA precipitate with 1.0 mL of ice-cold 70% ethanol. Wash again with 1.0 mL of ice-cold TE buffer. Be careful so as not to lose any of the DNA.

9. Allow the DNA to air dry. Re-suspend the DNA completely in 5.0 mL of TE buffer. This is your stock DNA solution. Label your sample and give to your instructor for overnight incubation at 37°C to re-dissolve the DNA.

Before the next lab period (preferably the next day)-

10. Your instructor will filter your re-dissolved DNA solution through a plug of glass wool into a sterile culture tube. And store your group’s DNA solution.

11. Using pipettes dilute 40.0 µL of your DNA stock solution with 40.0 µL TE buffer to make a 1 to 2 diluted solution. Remember to mix gently but thoroughly.

B. 1. Take the absorbance of 70.0 µL of your diluted DNA sample at 260 nm and 280 nm with the Beckman DU 640 spectrophotometer. If the A260 absorbance is above 1.0, or less than 0.5, make a new dilution from the
original stock solution that will be in the $A_{260}$ absorbance will be in the range of 0.5 - 1.0. Consult with your instructor first. Record your actual dilutions in your lab notebook.

2. Store your labeled, diluted sample and the remainder of your stock solution in the freezer until next lab.

3. Calculate the concentration of your diluted DNA solution in mg/mL and $\mu$g/$\mu$L using the $A_{260}$ values for the unheated solution. Use the fact that a 1.0 mg/mL solution of native duplex DNA gives an $A_{260} = 20.0$ at neutral pH in a 1.0 cm path length cuvette (i.e., $k = 20.0$ mL·mg$^{-1}·$cm$^{-1}$). Calculate the DNA concentration for the original, undiluted stock DNA solution.

4. Calculate the g DNA/mg bay scallop abductor muscle tissue based on the mass of your starting tissue.

5. Calculate the $A_{260}/A_{280}$ ratio for the unheated sample. Explain the meaning of these ratios with respect to purity.

6. Return your original undiluted stock DNA solution to your instructor; it will be placed in the freezer until the next lab.

IV. Points for Discussion:

For your progress report describe briefly the isolation procedure and the purpose of each step and reagent used. Report the DNA concentration in mg/mL and $\mu$g/$\mu$L for your original, undiluted, unheated DNA solution based on the $A_{260}$ extinction coefficient. What is the $A_{260}/A_{280}$ value for the DNA solution? What does this ratio suggest in terms of the purity of your isolated DNA? Finally report the $\mu$g DNA/mg scallop abductor muscle tissue. How does this value compare to that expected?

V. References:


