

## **Introduction**

DNA can be extracted from the tissues of a number of organisms essentially by:

- 1) breaking down the cell and nuclear membranes
- 2) separating out or degrading cellular proteins
- 3) precipitating out the DNA

These are the three basic steps in the procedure. Homogenization, deproteinization, and precipitation. The homogenization step involves grinding the dried sample in order to break down the cells. The tissue is mixed with the homogenization medium containing a non-ionic detergent (t-octylphenoxypolyethoxyethanol, a.k.a. Triton X-100), which breaks down the cell membrane, and membranes around vacuoles that contain stored protein, then denatures the proteins.

Deproteinization involves precipitation of starches and cell nuclei, and removal of the supernatant that contains denatured proteins and other soluble substances. DNA is separated from starch by the addition of an anionic detergent (sodium dodecyl sulfate, a.k.a. SDS) that dissolves the DNA, and allows the removal of starch in the precipitate. DNA is removed from the supernatant by adding ethanol that allows all the components to stay in solution except DNA. The DNA will gather at the interface of the solution and ethanol, and then is spooled out with a glass rod.

## **Solutions:**

- 1) 10 mM MgCl<sub>2</sub>, 10 mM Tris Buffer pH 7.5 and 0.2 M NaCl
- 2) Triton X- 100 ( 20% in H<sub>2</sub>O )
- 3) 10 mM MgCl<sub>2</sub>, 10 mM Tris Buffer pH 7.5 and 0.5 % Triton X- 100
- 4) 1 % Sodium Dodecyl Sulfate (SDS) solution in H<sub>2</sub>O
- 5) ice-cold 95% ethanol
- 6) Tris-EDTA buffer (TE buffer). (0.01 M in Tris-HCl and 0.001 M EDTA)

## Laboratory:

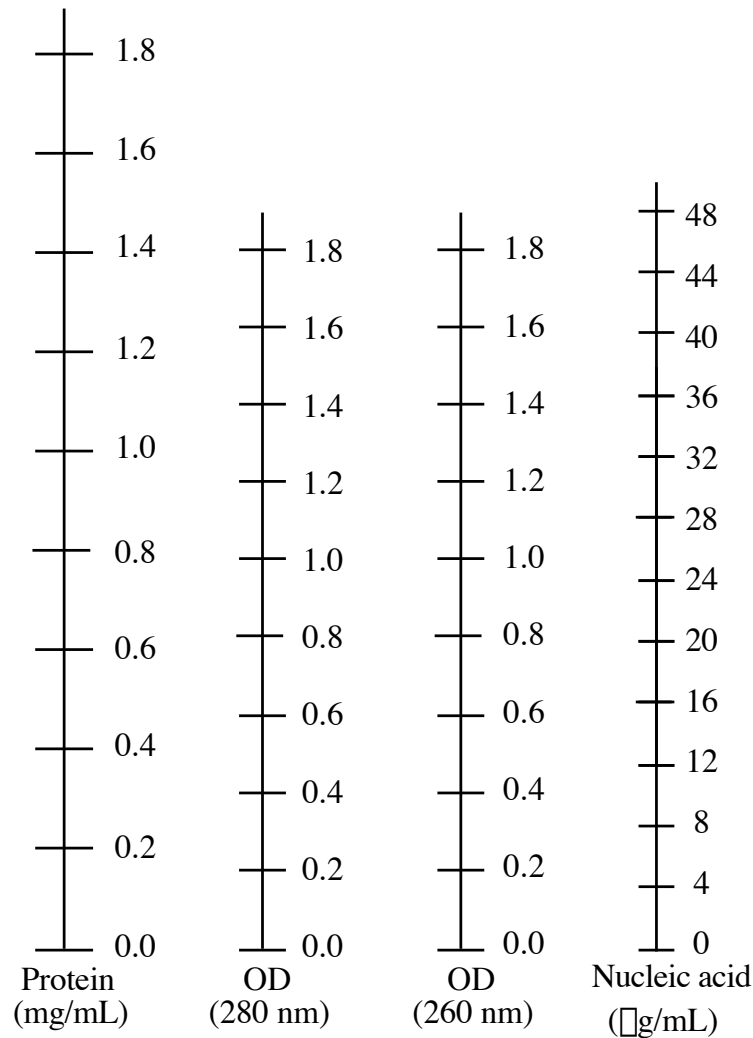
- 1) Grind approximately 1 g of dried pea seeds (dried onion or wheat germ also works) with a coffee grinder or similar. Add 10 mL of Solution 1.
- 2) Place this mixture in a small beaker and add 0.25 mL of Solution 2. Stir the mixture thoroughly for at least 45 minutes using a magnetic stirrer plate and magnetic stir bar.

NOTE: to considerably speed up the extraction procedure, your instructor will prepare a bulk mixture of sufficient volume for a whole class by scaling up the above volumes.

- 3) Filter the suspension through damp gauze in a filter funnel into a 15 mL centrifuge tube. (If bulk preparation was done, take and filter 10 mL of the solution.) Squeeze any liquid out of the filter gauze and throw away the residue.
- 4) Using a bench centrifuge, spin the filtrate until there is a well packed creamy pellet at the bottom of the test tube (about 5 minutes).
- 5) The liquid above the solid contains proteins and other soluble substances. Pour off this liquid and keep the pellet in the bottom of the tube, which consists of starch and cell nuclei.
- 6) Add about 10 mL of Solution 3. Stir gently with a glass rod until the pellet and the solution are completely mixed. Centrifuge again until a well-packed pellet is formed (about 5 minutes). Discard the supernatant liquid that will contain more protein.
- 7) Add 3 mL of Solution 4 and stir gently with a glass rod for several minutes to disperse all lumps. Centrifuge again until a well-packed pellet is formed. This pellet will be mainly starch and the DNA will be in the supernatant liquid.
- 8) Use a Pasteur pipette to draw off about 2 mL of the liquid and put in a clean centrifuge tube. Leave behind any foam on the top of the liquid and the pellet of starch.
- 9) Slowly add about 4 mL of very cold ethanol to the top of the liquid in the tube. Pour it down the inside surface of the tube with a Pasteur pipet so that the ethanol forms a layer on top of the DNA solution and does not mix with it.
- 10) Take a Pasteur pipet and hold the narrow end with forceps. Heat it with a Bunsen burner for a few seconds, carefully bend the end back up towards the top with forceps to form a hook, and remove from flame while still holding with forceps until cool.
- 11) Gently rotate the glass hook at the interface where the ethanol touches the DNA solution and observe the formation of the long fibrous strands of DNA. You should get the DNA to stick to the glass rod, and after spooling, pull it out of the test tube (this is best done by rotating at the interface first, then lifting the glass hook from the bottom of the DNA layer through the ethanol layer then into the air). DNA is fairly transparent. If your DNA appears whitish the color will be due to contamination with protein that was not completely removed.
- 12) To use the DNA for uv spectrophotometry, dry with filter paper and resuspend as much of the spooled DNA as possible in 5 mL TE buffer. Centrifuge for 2 minutes and determine absorbance at 260 nm and at 280 nm.

**Report:**

- 1) Write complete descriptions (including composition and function) of the following components of eukaryotic cells: cell membrane, cytosol, nucleus, ribosomes, lysosomes, and mitochondria. Use information from your textbook (Table 1.5 and 1.7; and in later chapters, see textbook Index), other books, the Web (Chem 365 Web page, under Biomolecule links) or notes from other classes to compose your descriptions. **You must cite the references you used in order to receive credit for this part of the report.**
- 2) Write down the complete structure for the tetradeoxynucleotide 5'ACTG3'.
- 3) Explain the purpose of the Triton X-100, NaCl, SDS, and ethanol in the preparation.
- 4) Prepare a solution of your product in TE buffer (□ 5 mL Solution 6) and find its absorbance at 260 nm and at 280 nm. Determine the concentrations of protein and nucleic acid in the solution from the nomograph below. Include a copy of the ultraviolet absorption spectrum with your report.
- 5) Calculate the weight percent of your DNA recovered relative to your starting sample. Clearly show the setup of your calculation.



## **DNA Physical Properties Related to Isolation**

**Reference:** Boyer, R.F. *Modern Experimental Biochemistry* Benjamin/Cummings: Menlo Park, CA, 1986.

### **1. pH**

- a) Complementary H-bonding stable between 4 - 10
- b) Phosphodiester linkages stable between 3 - 12
- c) N-glycosidic bonds hydrolyze below 3

### **2. Temperature**

- a) 80 - 90°C will start to separate strands
- b) Phosphodiesters and N-glycosides stable up to 100°C

### **3. Ionic Strength**

- a) DNA most stable in salt solutions above 0.1 M

### **4. Cellular conditions**

- a) Cell wall, plasma membrane and nuclear membrane must be broken
- b) Deoxyribonucleases must be deactivated
- c) Native DNA present as DNA-protein complexes; Proteins (histones) must be dissociated