

Name _____

Pre-lab 1–DNA Isolation

1. Arrange the following steps of DNA isolation into logical order by numerical assignment.
___ Removal of detergent foam and un-macerated tissue during filtration
___ Deactivation of enzymes that destroy DNA
___ Breakdown of cell walls, cell membranes, and nuclear membranes
___ Collection of DNA after treatment with ethanol
2. Sodium lauryl sulfate is capable of breaking apart (or dissolving) cell and nuclear membranes because its structure contains regions that are _____ as well as regions that are _____.
3. The chemical EDTA is included in the homogenizing medium because:
 - A. it makes the temperature optimal.
 - B. it breaks down the onion cell walls.
 - C. it chelates (binds) magnesium, disabling the enzymes that destroy the DNA in solution.
 - D. it aids in membrane break down and removes chromosomal proteins.
4. What chemical property of DNA causes it to be soluble in water and insoluble in high salt and ice-cold ethanol? _____
5. What chemical property of DNA causes it to be attracted to glass? _____

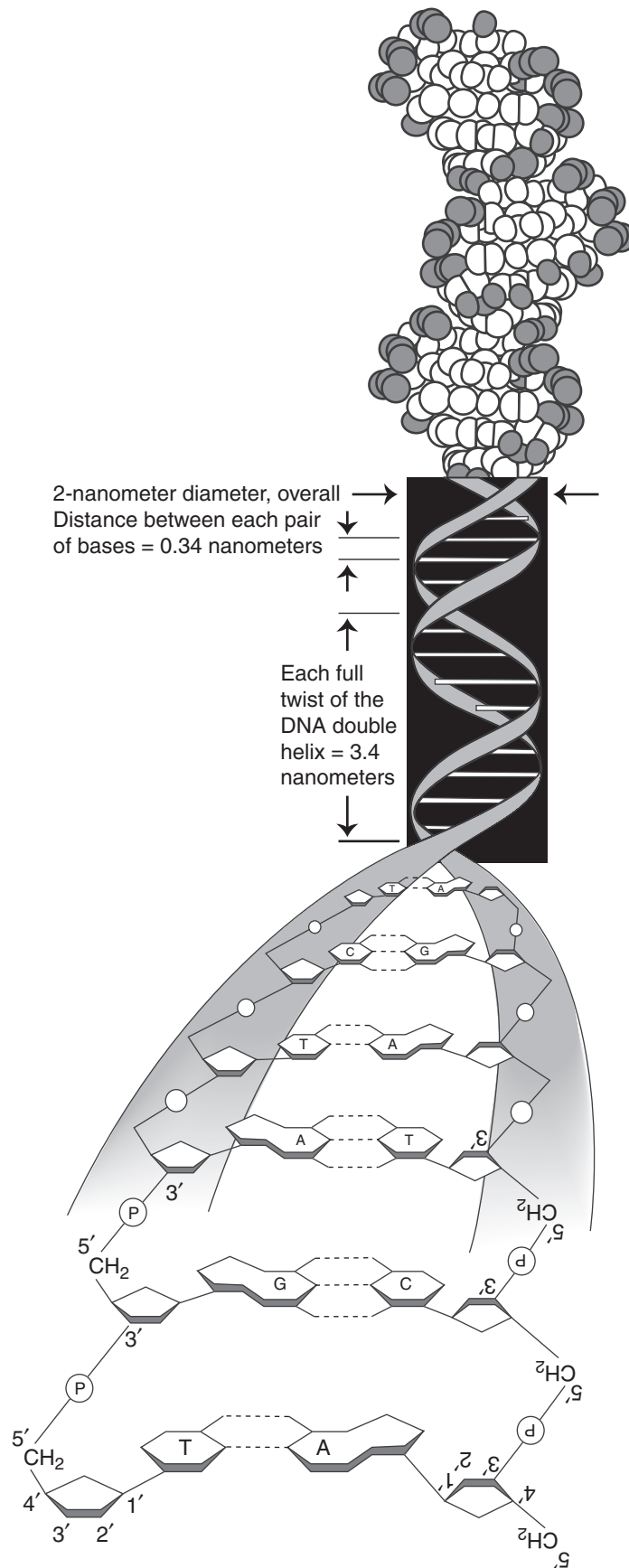


Figure 1.1 DNA

LABORATORY

Isolation of DNA From Onions; Gene Expression

BACKGROUND

The purpose of this laboratory session is to give you first-hand experience with DNA by isolating it from onion tissue. This should be an exciting experience. You will start with whole onions and end up with a relatively pure preparation of viscous DNA. Your sample of DNA will be large enough to contain literally billions of genes. We will keep this sample and use it in the following lab, DNA Analysis.

The information guiding the development according to one's ancestral kind resides in the genes found in DNA. To most students of biology, DNA is an abstraction at best. You can memorize the names and structures of the organic bases found in DNA, and know about the history of its discovery, but until you handle DNA yourself, it remains a strange and mysterious substance.

It is not the purpose of this laboratory introduction to repeat in great detail the points of DNA structure and function that are already covered in your text. This laboratory unit gives you your first opportunity to conduct a series of procedures leading to the isolation of a specific chemical compound from living cells. You should understand why each step is carried out.

A. Homogenization and DNA Solubilization

Whole cell DNA includes more than just DNA from the nucleus; organelles such as chloroplasts and mitochondria also contain DNA. In order for DNA to be released from the onion cells, we must destroy the cell walls, plasma membranes, and nuclear membranes. Destruction of the cell walls is accomplished by a combination of treatment with the components of the homogenization medium (5% SLS, 0.15 M NaCl, 0.001 M EDTA in 0.015 M sodium citrate, pH 7.4) and maceration of tissue in a blender. The homogenization medium, particularly the SLS, also destroys the cellular and nuclear membranes. SLS stands for sodium lauryl sulfate, a common detergent found in shampoos. The polar and nonpolar regions of SLS allow it to associate with the polar and non-polar regions of a phospholipid bilayer causing membranes to lyse or be pulled apart (dissolved). SLS also aids in the separation of chromosomal proteins from the DNA molecules.

DNA is easily degraded if strict attention is not paid to the isolation procedure. Extremes of pH and temperature, and low concentrations of ions should be avoided. Low pH (acidity) causes the breakage of bonds between the bases and the sugar, the phosphates and the sugar, and disrupts hydrogen bonds between complementary strands of the DNA structure. The hydrogen bonds are also susceptible to breakage under conditions of high pH (alkalinity) and low ionic concentration. Moderate temperatures increase the activity of DNA nucleases (a group of enzymes responsible for breaking down DNA), however these enzymes will denature at 60°C. Higher temperatures

than this are undesirable because of their destructive effect on some DNA bonds. The SLS and EDTA help prevent the action of the enzymes by denaturing the enzymes and chelating the Mg^{2+} cofactor, respectively.

B. Denaturation of Proteins (We will not use chloroform due to its toxic nature!)

In living cells, DNA is complexed with chromosomal proteins of various types. These proteins must be removed from the DNA before DNA can be isolated. Although SLS dissociates the chromosomal proteins from the DNA, it does not remove the proteins from the solution itself. If it is absolutely necessary to remove all proteins from the DNA solution (this depends on the type of experiment you are planning), a common technique for protein removal is to use chloroform ($CHCl_3$). Isoamyl alcohol (1 part per 24 parts of chloroform) is added to the chloroform to prevent frothing of the denatured protein. The chloroform-isoamyl alcohol mixture is not miscible in the aqueous homogenizing medium and forms a separate layer below it. The denatured protein collects at the interface between these two layers because proteins have regions of polarity that associate with the aqueous (water) solution, and regions of nonpolarity that associate with the chloroform mixture.

C. Precipitation of DNA

DNA is not soluble in ice-cold ethanol due to its nonpolar nature, especially when salt is present because it ties up the water molecules. Therefore, in your final isolation step, you will precipitate DNA by adding 1½ to 2 volumes of **ice-cold** ethanol to your preparation. If the DNA has not been partially degraded or denatured, it should precipitate as a thick, stringy, white mass at the interface between the extract and the ethanol. The precipitated DNA may be collected onto a clean glass rod by “spooling” the solution. If the DNA has been partially degraded, it will still precipitate, but as a flocculent white mass that will not spool onto the glass rod. Both the cold temperature and the nonpolar nature of the alcohol will help solidify the long strands of DNA.

Procedure

In this laboratory activity, you are to work in groups as designated by your instructor. Part of the reason for working in groups is so that you can each monitor the other's laboratory activities. Do not proceed to a subsequent step until all of you agree that it is permissible. One false step in this procedure could ruin your entire preparation (i.e., you may not be able to isolate any DNA).

Homogenization:

1. Each pair of students should start with half of a medium-sized onion. Using the available knife and cutting board, you should finely dice your onions. **Why?** There is a possibility that this step may have been done for you already to save time.
2. Obtain 100 ml of diced onion (approximately 50 g) and transfer all of this material to a 250 ml beaker.
3. To the diced onions in your beaker, add 100 ml of homogenizing medium. (Break pieces up)
4. Incubate your preparation in the 60°C water bath for at least 15 minutes. **Why** is this step necessary?
5. Cool your preparation to room temperature in an ice bath. Your solution should no longer be warm to the touch. This will take about 5 minutes.
6. Homogenize your cooled preparation in the Waring blender for 3 seconds at low speed, followed by 3 seconds at high speed. **Over-blending will shear your DNA sample.**
7. Pour the homogenate out of the blender and into a 600 ml beaker.

8. Filter the homogenate through four layers of cheesecloth into a 250 ml flask, taking care to leave the foam behind. Accomplish this task by taping the layers of cheese cloth over the top of the 600 ml beaker in a secure way. Then drain the homogenate into the 250 ml flask using a funnel to hold the 600 ml beaker with cheese cloth. Alternate method: Line a funnel with 4 layers of cheesecloth and place it in a 250 ml beaker. Pour the homogenate into the funnel and collect the filtered liquid.

Where do you suppose the DNA is at this point? Soap bubbles usually disperse after a few minutes. Your foam doesn't seem to do that. Can you think of any reasons why this might be so?

Precipitation of DNA:

1. Transfer 5 ml of homogenate to a test tube.
2. Place your test tube with homogenate in an ice bath.
3. Slowly add about 2 volumes (twice the amount of solution already in your tube) of ice-cold 95% ethanol by pouring it down the inside wall of the test tube. DO NOT MIX.
4. Slowly rotate a clean glass rod in the test tube, and so entangle the stringy DNA precipitate. Continue to spin your rod as you move it in circles through the test tube. It is important to spin your rod always in the same direction. **Why?** (This step should be done while the tube is in the ice bath.)
5. Gently ease the DNA off the end of the glass rod into the vial provided, and fill it with 50% alcohol for storage. If the DNA sample does not adhere to the glass rod you can use the pipette to withdraw a sample and place it into the vial. Be sure to tighten the screw cap to prevent leakage.

Dry load gels

While agarose gels are setting, students will practice loading samples with a micropipette on a preformed gel using loading solution only.

END OF CLASS DISCUSSION

Describe the appearance of the solution at each of these steps.

- A. After homogenization.
- B. After filtration.
- C. After ethanol has been added.

What substances are present in the aqueous homogenate after each of the following steps?

- A. After homogenization.
- B. After filtration.
- C. After ethanol has been added.

Describe the role of each of these in the isolation procedure.

- A. NaCl–
- B. SLS–

- C. DNA nucleases–
- D. 95% Ethanol–
- E. EDTA–
- F. Citrate Buffer–
- G. 60° incubation–

INVESTIGATION B: GENE EXPRESSION

Background

The objective of this experiment is to learn the importance of the expression of a single gene to the survival of an organism. In this experiment you will be working with a strain of *Escherichia coli* bacteria that is commonly used in recombinant DNA technology. One sample of this bacterium has been transformed by inserting a plasmid (small, circular section of DNA) into the cytoplasm of the bacteria. This plasmid carries a gene that codes for the production of a protein called Beta Lactamase. This enzyme degrades the antibiotic Ampicillin. When the bacterium has this plasmid, it is resistant to the action of this antibiotic.

You will take bacteria from two stock samples. One has been transformed with this plasmid and one does not have the plasmid (but you won't know which is which). Apply both cultures to each of two growth plates: one is a control plate of nutrient agar and the other is the experimental plate containing nutrient agar and ampicillin.

Your instructor will demonstrate the proper technique for transferring the bacteria to your plates. The plates will be incubated at 37°C until growth occurs and you will analyze them for growth. **BE SURE TO WASH YOUR HANDS THOROUGHLY AFTER PLATING THE BACTERIA.**

Materials

control agar plate
stock control bacteria
sterile cotton swabs

experimental agar plate (+ampicillin)
stock transformed bacteria

Procedure

Day 1 (The current lab week)

1. Make a line through the diameter of the control and the experimental plates. Label the plates either CONTROL or EXP (for experimental) and with your lab group name. **HINT:** always label the bottom of the plates (the part holding the culture medium). The tops can get switched around and then it becomes impossible to interpret your results!
2. On each plate, label one half "STRAIN 1" and the other half "STRAIN 2."
3. Take a cotton swab and carefully remove a sample of the bacterium from the Strain 1 culture (be careful not to contaminate the swab).
4. Lightly streak the swab on the STRAIN 1 side of both plates. Your instructor will demonstrate.

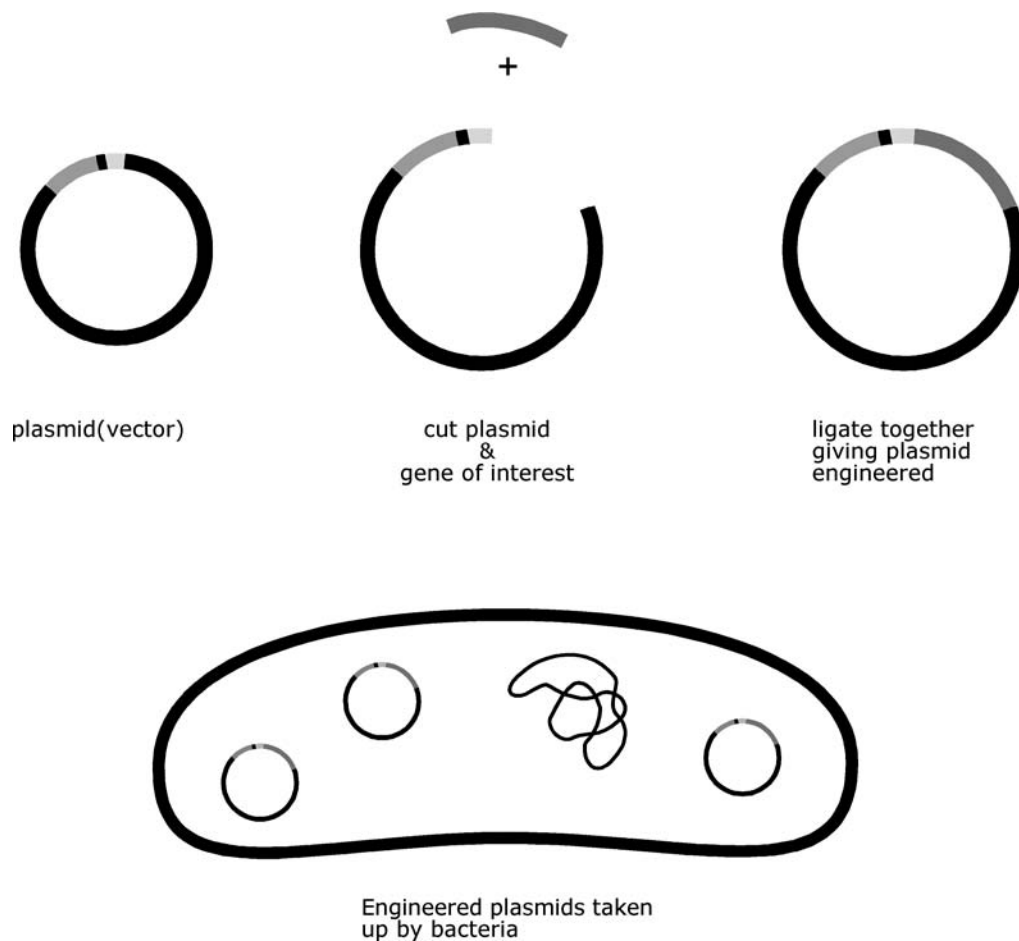


Figure 1.2 Transformation of *E. coli* with a plasmid containing the insulin gene.

5. Repeat with the sample from the stock bacterium labeled Strain 2, streaking the STRAIN 2 side of both plates.
6. Give the plates to your instructor to incubate overnight at 37°C. The instructor will then store the plates in the refrigerator so that you can evaluate growth **at the beginning of the next lab session**, and determine which of the strains contains the plasmid with the ampicillin-resistance gene.

Day 2 (next week) – See Lab 9 Part C for instructions.