

**Molecular Biology Lab**  
**Pre-Lab Exercise**

Name \_\_\_\_\_

1. What are the three different chemical parts of a nucleotide? Draw a simple sketch to illustrate how the three parts are arranged.
2. What are the 4 bases that make up DNA? What are the base-pair rules?
3. What type of chemical bond holds nucleotides together to form a strand of DNA? What type of chemical bonds hold the two DNA strands together?
4. What does denaturation mean when applied to DNA?
5. How does gel electrophoresis separate molecules of DNA?

# Molecular Biology Lab

## Work in groups of four

In today's lab, we will examine some of the physical properties of DNA and demonstrate some of the techniques that are used to visualize and analyze DNA.

### Summary of Activities

1. Examine the models of DNA.
2. Examine the physical properties of salmon sperm DNA.
3. Isolate DNA from onion.
4. Analyze the DNA using gel electrophoresis.
5. Act out protein synthesis using the protein synthesis game.
6. Streak agar plates with environmental swabs for observation next week.

### §

DNA is a molecule that can be extremely long, but is also extremely thin and easily broken. Even though there is approximately 6 feet of DNA in each cell in your body, its thread-like thinness enables it to be wound up and stored in a very small space, the nucleus. Because of this thinness, it cannot be visualized directly. We can, however, use a variety of techniques to visualize DNA indirectly.

#### I. DNA Model:

1. Identify the sugar-phosphate backbone on each strand.
2. Observe the base pairing. You can easily identify the G-C pairs because they have three hydrogen bonds, whereas A-T pairs have only two.
3. Note the double helix.

#### II. Salmon Sperm DNA

##### Materials

- 1 ml pipet
- TE buffer
- glass pipet for spooling
- 100 mg salmon sperm DNA
- 2M NaCl solution
- Ice cold 95% ethanol

1. Examine your piece of purified DNA. Describe its appearance and texture.

2. Add 3 ml of TE buffer to the DNA in the test tube.
3. Gently shake the mixture to dissolve the DNA. **DO NOT USE THE PIPET TO STIR THE DNA!** Get a feeling for the viscosity of the dissolved DNA by tipping the tube from side to side (with the cap on). **Viscosity** refers to the thickness, or goopiness, of the solution. The more viscous a solution, the thicker and goopier it is. It usually takes about 10 minutes to completely dissolve the DNA. Record your observations.
4. Add 0.25 ml of the 2M NaCl solution (table salt) and mix gently.
5. Slowly add 3 ml of **ICE COLD** 95% ethyl alcohol so that it runs down the side of the tube, rather than dripping directly into the DNA solution. You may see the alcohol form a layer on the top of the DNA solution.
6. Gently stir your solution with the glass pipet using an up and down motion to mix the two layers. You should see the DNA precipitate out of solution, forming thin white strands. Swirl the stirring rod around so that the DNA wraps around the stirring rod, like winding spaghetti on a fork. This is called "spooling out" the DNA.

DNA is not soluble in the presence of salt and alcohol. When these two chemicals are added to a solution of DNA, the DNA becomes a thread-like solid.

7. Describe the appearance of the DNA during this process.

- Q1. Why does the dry Salmon sperm DNA look so much like thread or string?*
- Q2. Why is DNA in solution so viscous?*
- Q3. What does it mean for the DNA to "precipitate out of solution"?*

Q4. Why does the DNA spool around the glass rod?

### III. Isolating DNA from onion:

#### Materials

- 1/4 yellow onion
- Two 100 ml beakers
- Glass stir rod
- Funnel
- Cheesecloth
- Knife
- Cutting board
- Balance
- 25 ml homogenizing medium
- 1.25 g sodium dodecyl sulfate (SDS)
- 95% ethanol, ice cold
- 3 or 4 – 50 ml flasks, with pipets
- 60°C water bath
- Thermometer
- Ice bath
- Mini food processor
- 3 ml graduated pipet for dispensing onion homogenate

In this exercise, we will start with a whole onion and isolate its DNA. One onion contains miles of DNA and billions of genes!

The steps in Part A prepare the onion for DNA extraction. In order to get the DNA out of the cells, the cell walls, plasma membranes, and nuclear membranes must be broken down. Next, the DNA will be separated from some of the proteins that are bound to the DNA in the chromosomes. These first steps will be divided among the student groups.

In Part B, each group will precipitate the DNA from a portion of the prepared onion mixture.

#### Part A: Onion Preparation

1. Peel and dice 1/4 onion.
2. Weigh out 12 g of diced onion and place in the jar of the mini food processor.
3. Add 25 ml homogenizing medium to the onion in the mini food processor and put the lid on. Process the onion on medium high for about 1 minute. The homogenizing medium contains salts that help maintain the structure of the DNA during the isolation process.

Q5. What does the mini food processor do to help get the DNA out of the cells?

4. Pour the processed onion mixture into a 125 ml flask. Add 10 ml sodium dodecyl sulfate (SDS) and mix well with a glass stir rod. SDS is a detergent that helps dissolve cell membranes and denature proteins.
  5. Heat the flask in a 60°C water bath for 12–15 minutes; remove promptly and place the beaker into an ice bath. The heat softens the onion tissues, allowing the SDS and homogenizing medium to penetrate.
- Q6. There are a number of enzymes present in the nucleus that could interfere with the DNA isolation process. What does the heat treatment do to prevent this interference?
- Q7. Once the SDS is added, the mixture is referred to as a “lysate”. Why?
6. Place the flask in the ice bath until it reaches room temperature (about 5 minutes). Check the temperature by periodically feeling the outside of the flask. At room temperature, it should feel slightly cool. Cooling the lysate prevents **denaturation** of the DNA, in which the hydrogen bonds holding the two strands together are broken.
  7. Filter the lysate using a funnel and 4 layers of cheesecloth into a clean 125 ml flask, keeping the flask on ice if possible. It may take several minutes for the lysate to go through the cheesecloth.

#### Part B: Spooling the DNA

To be done by each group.

1. Transfer 4 ml of the onion lysate to a clean test tube. Swirl your spooling pipet in the lysate to get an idea of its texture. Note the color as well. Rinse and dry the spooling pipet before proceeding to the next step.
2. Slowly add about 2 ml of ice cold 95% ethanol down the side of the test tube as you did with the salmon sperm DNA. The ethanol will form a distinct, clear layer over the yellowish onion lysate. As you add the ethanol, you will notice a new layer forming between the ethanol and the onion lysate. As with the salmon sperm DNA, it

should be clear and slightly jelly-like, with tiny whitish strands. This layer is the onion's DNA!

3. Gently swirl the end of the spooling pipet around in the DNA layer so the DNA wraps around the pipet.
4. Describe the appearance and texture of the DNA before and after adding the ethanol. Compare your observations to the salmon sperm DNA.

*Q8. How would the results of this procedure be affected if the SDS was not added? Explain your answer.*

#### **IV. Gel Electrophoresis:**

The size of a piece of DNA can be analyzed by using a technique called gel electrophoresis. In this technique, a piece of DNA is cut into very specific sizes using enzymes called **restriction enzymes**. Restriction enzymes recognize specific sequences of DNA and will always cut in the same place. After the DNA has been cut using the enzymes, the DNA solution is loaded into a jello-like substance called agarose.

The agarose gel is then subjected to an electric field that will pull the DNA to the opposite side of the gel. The DNA will move because its ionic properties will cause the DNA to be attracted to the positive charge of the electric field.

The agarose acts somewhat like a molecular obstacle course for the DNA. The pieces of DNA that are smaller and more agile will be able to travel through the agarose quicker than larger pieces. When we turn off the electrical field and look at the DNA in the gel, the DNA will be separated by size. Smaller pieces of DNA will be closest to the bottom, with larger pieces of DNA closer to the top.

1. Observe the demonstration loading the DNA sample onto the gel, and how the electric current pulls the dyes added to the DNA through the gel.
2. Observe the specially stained agarose gel that has been previously prepared. Observe the distribution of DNA.

*Q9. What do you think would happen if the electrodes were reversed so that the negative charge of the electric field is at the bottom of the gel and the positive charge is at the top?*

*Q10. What are some of the uses of analyzing DNA in this manner?*