1. What are the three chemical parts of a nucleotide. Draw a simple sketch to show how the three parts are arranged.

2. What are the rules of base pairing?

3. In double stranded DNA, what type of chemical bond holds the two strands together?

4. What does denaturation mean when applied to DNA?

5. How does gel electrophoresis separate molecules of DNA?
Molecular Biology of DNA

Work in groups of two and bring your textbook

In today’s lab we will examine how cells use DNA to make proteins, examine some physical properties of DNA, and demonstrate some of the techniques used to visualize and analyze DNA.

Objectives
1. Examine the molecular structure of DNA.
2. Practice the cellular process of DNA replication and protein synthesis.
3. Isolate DNA from onions.
4. Observe gel electrophoresis.

Part I: DNA

A: DNA Structure
The phenotype of each person depends on the DNA they inherit from their parents. DNA is a double-stranded molecule resembling a twisted ladder. Each strand is composed of a long chain of molecules called nucleotides. Conversely, each nucleotide is composed of three parts: a five-carbon sugar (deoxyribose), a phosphate group, and a nitrogen base. There are four different nitrogen bases in DNA:

- Adenine (A), Thymine (T), Cytosine (C), and Guanine (G)

Each side of the DNA ladder consists of a chain of phosphate and sugar molecules, and the rungs are made of two bases held together by hydrogen bonds (a weak attraction between hydrogen and oxygen atoms). Note that in forming the rungs, A is always paired with T, and C is always paired with G.

1. Examine the three-dimensional model of a DNA molecule and identify the sugar-phosphate backbone, the nitrogen bases, and the hydrogen bonds that link the two complimentary strands.

Q1. Describe how the two strands of the DNA helix are oriented and why A pairs with T and C pairs with G?

2. Draw a two-dimensional representation of a segment of DNA. Each strand should consist of 5 nucleotides. You can represent the different parts of each nucleotide using the letters below:

- S = sugar (deoxyribose)
- T = thymine
- P = phosphate
- A = adenine
- C = cytosine
- G = guanine
B: DNA Replication

During interphase of cell division we have learned that the DNA in a cell is duplicated. In this way every new cell will have an exact copy of the original cell’s genetic material. This is accomplished when each strand of DNA serves as a template to form a new complementary strand. The process is called replication.

1. **Draw a segment of DNA undergoing replication** (refer to text pages 190-191). Have your DNA contain 14 base pairs with half of the molecule unzipped and replicated. Label parental strands and daughter strands, the replication fork, the enzymes DNA polymerase and DNA ligase. Be sure that template bases appropriately match the bases of both new strands. **Use may use a different colored pencil/pen to draw the daughter strands**.

Q2. Assume a single error occurs in the complementary base pairing process of DNA replication: a) What do you think the effect would be on the cell where the error occurred? B) If this mutation were to occur in one of your body cells, could your children inherit it? Explain.

Part II: How Cells Make Proteins

The process for making protein can be summarized as follows:

DNA **transcription** > mRNA **translation** > protein
code codon product

A: Transcription

DNA serves as a template for making RNA. Thus RNA contains the instructions for making a protein. Unlike DNA that cannot leave the cell’s nucleus, RNA can enter the cytoplasm where proteins are made. Also, RNA has a different sugar (ribose); it substitutes uracil (U) for thymine; and it is a single strand.
The nucleotide bases in mRNA are complementary to the nucleotide bases in DNA. In mRNA, sequences of 3 nucleotide bases serve as codes for single amino acids and are called **codons**. The strands of mRNA are formed by the process called **transcription**, since they are transcripts of the DNA. The mRNA leaves the cell nucleus and enters the cytoplasm with the instructions for what amino acids will be needed to assemble a specific protein.

1. The figure below shows a portion of a DNA molecule whose strands have been separated by an enzyme for the synthesis of mRNA (transcription). The bases are shown for the strand that is NOT serving as the template.

   a. First, write in the bases on the DNA strand that is serving as the template.
   b. Second, write in the bases of the newly formed mRNA molecule.

   ![DNA and mRNA diagram]

2. Use the following DNA code to **construct an mRNA transcript**.


   mRNA transcript: ________________________________

   **Q3. What is the purpose of making a mRNA transcript and how is the molecule different from the DNA template?**

   **B: Translation**

   RNA exists in three distinct forms: messenger RNA (**mRNA**), ribosomal RNA (**rRNA**), and transfer RNA (**tRNA**). The three types of RNA work together to synthesize proteins. The process of interpreting the DNA code to make a protein is called **translation**.

   Assembling a protein begins with each tRNA transporting a single amino acid to the ribosomes of a cell. There the tRNA **anticodons** (triplets of bases complementary to codons) are matched one at a time with the mRNA codons contained in the transcript. The amino acids are then joined with peptide bonds to form the desired protein. As the amino acid from each tRNA is removed, the tRNA returns to the cytoplasm to pick up a replacement amino acid so that the process can be repeated again and again.
1. Using the following mRNA transcript,
   a. draw vertical lines to indicate the codons of the mRNA
   b. write in the bases of the tRNA anticodons
   c. identify the amino acids that will be assembled into a protein. Use the table on page 194 of the text to convert mRNA codons into amino acids.


tRNA anticodons: ________________________________________________________________

Amino acids: ________________________________________________________________

2. Use the mRNA codon---» amino acid conversion table (below) to complete the following:

<table>
<thead>
<tr>
<th>DNA</th>
<th>mRNA</th>
<th>tRNA</th>
<th>Amino Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TGA</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Q4. If a gene contained 3000 nucleotides, how many amino acids would be in the polypeptide that it codes for? (Explain your answer)

Part III: Isolating DNA from onion.

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

In this exercise, we will start with a whole onion and isolate its DNA. One onion contains miles of DNA and billions of genes!
In Part A, the onion is prepared 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 steps in Part A will be divided among the student groups.

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

**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 1.25 g 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?*

6. Place a thermometer into the flask and let the lysate cool in the ice bath until it reaches 15–20˚C (about 5 minutes). When checking the temperature of the lysate, raise the thermometer slightly so it is suspended in the lysate and not touching the bottom of the flask. Cooling prevents **denaturation** of the DNA, in which the hydrogen bonds holding the two strands together are broken.

   **Q7.** *Why would your temperature reading be inaccurate if you didn’t raise the thermometer up from the bottom of the flask?*

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.

**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.

Part 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. How is the DNA cut into pieces of such distinct size?

Q10. 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?

Q11. What are some of the uses of analyzing DNA in this manner?