1. What is DNA? Why is DNA important?

In this activity, you will extract DNA from the archaeon, *Haloferax volcanii*. You will be able to see the *Haloferax* as red growth on an agar plate. This growth contains many, many *Haloferax* cells.

In nature, *Haloferax* grow in extremely salty environments such as the Great Salt Lake, Dead Sea, or the very salty brine that results when seawater is evaporated to produce salt. To balance the high salt concentration in the surrounding environment, *Haloferax* cells have a high concentration of salt inside the cell.

2. The first step in extracting the DNA from the *Haloferax* cells will be to add water to the *Haloferax* on the agar plate. Based on your understanding of cells and osmosis, what do you think will happen when the *Haloferax* cells are put in water without salt?

- Use a pipette to add 5 mL of water to the plate of *Haloferax*. Use a Q-tip to gently move all the burst cells off the agar. Mix thoroughly.
- Each student should use a pipette to suck up 1 mL of the water with dissolved cell contents. You should be able to see the strands of DNA swirling as you suck up this solution. Notice that these long DNA strands make the solution viscous or goopy. Put the 1 mL of solution in your test tube. Before the fourth student in your group gets solution, you may need to add 1 mL more of water to the plate and stir.
- To extract the DNA from the water and other molecules in the solution, you will add a layer of chilled alcohol on top of this solution. Get a pipette with 1 mL of chilled alcohol.
- Tilt the test tube at a 45° angle, hold the pipette against the side of the test tube, and gently squeeze the pipette so the alcohol flows down the side and forms a layer on top of the water with dissolved *Haloferax* DNA. Do not shake the test tube. Gently place it where it can be undisturbed for 20 minutes while the DNA precipitates out of solution.

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1 By Drs. Ingrid Waldron, Lori Spindler, Jennifer Doherty and Mecky Pohlschroder, Dept Biology, University of Pennsylvania, © 2017. This Student Handout (a Word file which can be used to prepare a modified version if desired) and Teacher Notes (with instructional suggestions, background biology, and suggested alternative and follow-up activities) are available at http://serendip.brynmawr.edu/sci_edu/waldron/#dna.
While you're waiting for the DNA to precipitate, read the information below and answer questions 3 and 4.

DNA is a long molecule that consists of two strands of nucleotides twisted together in a long spiral called a double helix. DNA is made up of four different types of nucleotide: A, C, G and T.

Each DNA molecule contains multiple genes. Each gene is a segment of DNA with a sequence of nucleotides that provides the instructions for making a protein.

A cell needs many different types of proteins to function. For example, a cell needs:

- protein enzymes to carry out the chemical reactions needed for life
- transport proteins to move ions and molecules into and out of the cell and to move substances around inside the cell
- structural proteins.

3. All living organisms, including Haloferax, other microorganisms, plants, humans and other animals, are made up of one or more cells. Explain why all living organisms need to have DNA. Include the words genes and proteins in your explanation.

Genes influence an organism's characteristics by determining which types of proteins the organism makes. This flowchart shows an example in humans.

<table>
<thead>
<tr>
<th>DNA</th>
<th>→</th>
<th>Protein</th>
<th>→</th>
<th>Characteristic</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image.png" alt="DNA" /></td>
<td>→</td>
<td><img src="image.png" alt="Protein" /></td>
<td>→</td>
<td><img src="image.png" alt="Characteristics" /></td>
</tr>
</tbody>
</table>

One version of the gene gives instructions to make normal protein enzyme. → Normal enzyme makes the pigment molecule in skin and hair. → Normal skin and hair color

The other version of this gene gives instructions to make defective enzyme. → Defective enzyme does not make this pigment molecule. → Albinism (very pale skin and hair)

4. How do the two different versions of the gene result in normal skin and hair color vs. albinism?
20 minutes or more after you added the alcohol to your test tube, carefully examine the tube without bumping it. You should be able to see a translucent layer with DNA between the solution and the alcohol; often there will be strands of DNA stretching up into the alcohol, sometimes with bubbles on the strands.

Gently tilt the test tube at a 45° angle, put your stick ½ inch into the solution, and stir gently in one direction only to wind the DNA onto the stick. Then slowly draw the stick up along the inside of the test tube. You should see trailing goopy strands – this is the DNA.

Gently rub the stick on the edge of the test tube and stretch the stick outward slowly. You should see the DNA stretching out between the stick and the test tube.

**DNA Structure and Function**

This drawing shows a short section of a DNA double helix with a diagram of four of the nucleotides in each strand of the double helix. Each nucleotide has:

- a sugar molecule and a phosphate group (P) in the backbone of the DNA strand
- one of the four bases (A = adenine, C = cytosine, G = guanine, or T = thymine)

![DNA structure diagram](image)

Each base in one strand of the DNA double helix pairs with a base in the other strand of the double helix. The base-pairing rules describe which bases pair together in a DNA double helix. Complete the following sentences to give the base-pairing rules.

5. **A** in one strand always pairs with _____ in the other strand.
   **C** in one strand always pairs with _____ in the other strand.

Since all the nucleotides in DNA are the same except for the base they contain, each nucleotide is given the same symbol as the base it contains (A, C, G, or T).

6. A polymer consists of many repeats of a smaller molecule.

   DNA is a polymer of ____________________________ .

   A protein is a polymer of ____________________________ .
The sentence in this box summarizes how genes influence an organism’s characteristics.

The sequence of nucleotides in the DNA of a gene determines the sequence of amino acids in a protein which determines the structure and function of the protein which influences the characteristics or traits of the organism.

7. Explain how a difference in the sequence of nucleotides in a gene could result in one of these boys being albino and the other boy having normal skin and hair color.

DNA Replication

Our bodies need to make new cells to grow or to replace damaged cells. New cells are formed by cell division which occurs when a cell divides into two daughter cells. Before a cell can divide, the cell must make a copy of all its DNA; this is called DNA replication.

8. Explain why a cell needs to replicate its DNA before the cell divides into two daughter cells.

During DNA replication, the two strands of the DNA double helix are separated. Each old strand provides the information needed to make a new matching strand. Each nucleotide in the new strand is matched to a nucleotide in the old strand using the base-pairing rules.

The enzyme DNA polymerase helps to make each new matching strand. DNA polymerase:

- adds matching nucleotides one-at-a-time and
- joins each new nucleotide to the previous nucleotide in the growing DNA strand.

DNA replication results in two new DNA molecules that are identical to the original DNA molecule.
This drawing shows a short segment of DNA which separates into two strands in preparation for replication.

- Your job is to play the role of DNA polymerase and create the new matching strands of DNA to produce two pieces of double-stranded DNA. Add matching nucleotides one-at-a-time, using the base-pairing rules and the nucleotides and tape provided by your teacher.

9. Do both of these two new double-stranded pieces of DNA have the same sequence of nucleotides as the original double-stranded piece of DNA?
   yes ___  no ___

10. Why is it important that both copies of the DNA molecule have the exact same sequence of nucleotides as the original DNA molecule?

11. Based on the function of DNA polymerase, explain why each part of the name DNA polymerase (DNA, polymer, -ase) makes sense.

12. Explain how the double helix structure of DNA, the base-pairing rules, and DNA polymerase work together to produce two identical copies of the original DNA molecule.