Teacher Preparation Notes for DNA

This hands-on, minds-on activity includes an easy method for extracting DNA from the archaean, *Halofex volcanii*. Students learn about DNA structure, function and replication through analysis and discussion questions and hands-on modeling of DNA replication.

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Learning Goals
In accord with the Next Generation Science Standards:

- Students will gain understanding of the Disciplinary Core Ideas:
  - LS1.A, Structure and Function, "All cells contain genetic information in the form of DNA molecules. Genes are regions in the DNA that contain the instructions that code for the formation of proteins."
  - LS3.A, Inheritance of Traits, "Each chromosome consists of a single very long DNA molecule, and each gene on the chromosome is a particular segment of that DNA. The instructions for forming species' characteristics are carried in DNA."
- Students engage in the Scientific Practices, constructing explanations and using models.
- This activity provides the opportunity to discuss the Crosscutting Concept, "Structure and Function".
- This activity helps to prepare students for two Performance Expectations:
  - HS-LS1-1, "Construct an explanation based on evidence for how the structure of DNA determines the structure of proteins which carry out the essential functions of life..."
  - MS-LS3-1, "Develop and use a model to describe why structural changes to genes located on chromosomes may affect proteins and may result in harmful, beneficial, or neutral effects to the structure and function of the organism."

Specific Learning Goals

- DNA carries the genetic information in all types of living organisms. Each DNA molecule contains multiple genes.
- DNA consists of two strands of nucleotides wound together in a spiral called a double helix. Each nucleotide is composed of a phosphate group, a sugar molecule, and one of four different nitrogenous bases: adenine (A), thymine (T), guanine (G), or cytosine (C). The

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1 By Drs. Ingrid Waldron, Lori Spindler, Jennifer Doherty and Mecky Pohlschroder, Department of Biology, University of Pennsylvania, © 2016. These Teacher Preparation Notes and the Student Handout are available at http://serendip.brynmawr.edu/exchange/waldron/dna

2 A similar activity, but with DNA extraction from students’ cheek cells is available at http://serendip.brynmawr.edu/exchange/waldron/dna. An analysis and discussion version of the activity is available at http://serendip.brynmawr.edu/exchange/bioactivities/DNA.

The phosphate and sugar parts of the nucleotides form the backbone of each strand in the DNA double helix.

- The bases extend toward the center of the double helix, and each base in one strand is matched with a complementary base in the other strand. In accord with the base-pairing rules, A pairs with T and G pairs with C.

- A polymer consists of many repeats of a smaller molecule (a monomer). DNA is a polymer of nucleotides.

- Proteins are polymers of amino acids. The specific sequence of amino acids determines the structure and function of the protein. Proteins have many important functions in cells, including protein enzymes that catalyze chemical reactions, transport proteins, and structural proteins.

- The sequence of nucleotides in a gene gives the instructions for the sequence of amino acids in a protein. A difference in the sequence of nucleotides in a gene can result in a different sequence of amino acids which can alter the structure and function of the protein. This, in turn, can result in different characteristics, e.g. albinism vs. normal skin and hair color.

- DNA replication produces two new DNA molecules that have the same sequence of nucleotides as the original DNA molecule, so each of the new DNA molecules carries the same genetic information as the original DNA molecule. During DNA replication, the two strands of the original DNA double helix are separated and each old strand is used as a template to form a new matching DNA strand. The enzyme DNA polymerase adds nucleotides one-at-a-time, using the base-pairing rules to match each nucleotide in the old DNA strand with a complementary nucleotide in the new DNA strand.

**Supplies and Preparation for DNA Extraction**

In the following, we have described the amount of supplies needed if each student does his or her own DNA extraction. If your resources are limited, you can decrease the amount of supplies needed by having each group of students do a single DNA extraction; if you decide to do this, you will need to make appropriate changes in the instructions on page 1 of the Student Handout.

- *Halofex volcanii*. We have chosen the halophile, *Halofex volcanii*, for this activity because it is harmless and DNA extraction from halophiles is particularly easy. Moreover, because the agar for growing *Halofex* has a very high salt concentration, very few microorganisms and no pathogens will grow on this agar, so you do not have to observe sterile procedures and the plates may be disposed of without special precautions.

You can purchase a plate (slant) containing *Halofex volcanii* from Nasco ([https://www.enasco.com/product/Z50319M](https://www.enasco.com/product/Z50319M) or for 12 plates ([https://www.enasco.com/product/Z50320M](https://www.enasco.com/product/Z50320M)). This culture can last three months, but it is probably best to use a Q-tip or a spreader to streak the *Halofex* onto a fresh agar plate every 6-8 weeks if you are not going to do the activity within that time. Rub your spreader gently on the plate with *Halofex* and then spread the *Halofex* onto the new plate. Make sure to cover the whole plate. The plate should be stored in a plastic bag (to prevent drying out) upside down with the lid on the bottom (to prevent moisture from accumulating on the agar). The *Halofex* should grow for approximately 2-3 weeks at room temperature (or 3-5 days at 40-45°C if you have an incubator4). Each culture plate will provide enough *Halofex* for you to prepare at least 20 plates for classroom use. Each plate for classroom use should provide enough *Halofex* DNA for four students.

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4 An incubator is optional, but helpful. You can make a suitable incubator from a Styrofoam cooler (~30 cm high and 30 x 45 cm area at the top) with the bottom part lined with a large heating pad (~30 x 59 cm) and something like a tissue box on top of the heating pad to serve as a support for the plates. Do not let the temperature get above 45°C since this will inhibit the growth of *Halofex*. 
To grow the *Haloferax* for classroom use, use the procedure described in the previous paragraph. Leave the plates for approximately 2-3 weeks at room temperature (or 5 days at 40-45°C if you have an incubator). Once the plate turns red, the *Haloferax* are in a stationary phase; it is best to use the plates for the student activity within 4-6 weeks after that (or up to 8-10 weeks if you keep the plates in the refrigerator).

- **Agar plates** For each class, the number of agar plates you will need equals the number of groups of four students in that class. You may also want additional plates to grow the *Haloferax* for future use.

Obviously, you will need petri dishes. Possible sources include:
- https://www.amazon.com/SEOH-Petri-Dish-Sterile-Vented/dp/B0015T0LZO/ref=sr_1_1?ie=UTF8&qid=1479133263&sr=1-1

You can make suitable agar medium using one of the following recipes. You will be able to pour three plates per 100 milliliters of H₂O.

| Table 1. Agar recipe using mainly ingredients that can be obtained from a grocery store |
|-----------------------------------|------------------|
| **Ingredient**                    | **g/100 ml of H₂O** |
| Bacto™ Tryptone, Pancreatic Digest of Casein by Becton, Dickinson and Company | 0.5 |
| Difco™ Agar, Granulated, Solidifying Agent by Becton, Dickinson and Company | 1.5 |
| Morton Salt without Iodide        | 15 |
| Relief MD Epsom Salt (Unscented)  | 5 |
| Nu-Salt by Cumberland             | 1.0 |
| Regular Strength Antacid- Peppermint Flavor Calcium Rich- Shoprite Brand: Active Ingredient: Calcium Carbonate 500 mg (TUMS) | Crush half of the calcium pill with mortar and pestle |

| Table 2. Laboratory grade agar recipe (Tripepi et al. 2010[^5]) |
|-----------------------------------|------------------|
| **Ingredient**                    | **g/100 ml of H₂O** |
| Bacto™ Tryptone, Pancreatic Digest of Casein by Becton, Dickinson and Company | 0.5 |
| Bacto™ Yeast Extract, Dickinson and Company | 0.3 |
| Difco™ Agar, Granulated, Solidifying Agent by Becton, Dickinson and Company | 1.5 |
| NaCl                               | 12.5 |
| MgCl₂.6H₂O                         | 4.5 |
| MgSO₄.7H₂O                         | 1.0 |
| KCl                                | 1.0 |
| CaCl₂.2H₂O                         | 0.134 |

[^5]: Tripepi, M. S. Imam and M. Pohlschroder. *Haloferax volcanii* Flagella Are Required for Motility but Are Not Involved in PibD-Dependent Surface Adhesion
While stirring, carefully bring the water, tryptone and agar (for Laboratory grade recipe also yeast extract) to a boil in a flask that is at least twice the volume of the water to avoid superheating. Once the agar is completely dissolved, add the salts and return to the hot plate. When this medium is boiling, quickly remove it from the hot plate to prevent the liquid from boiling over. Repeat this process at least three times until the mixture appears clear and the salt is completely dissolved. At this point any salt-loving microorganisms that were associated with the salt should have been killed. Stirring continuously at slow speed, let the media cool down to 50-60°C before pouring the plates. Use at least 30 ml per plate to avoid drying out of the plate, since *H. volcanii* needs at least 4 days to grow.

After you pour the plates, they should be stored upside down in plastic bags and kept in a refrigerator. Because of the high salt concentration of the agar, potentially infectious organisms cannot grow on these plates so they may be disposed of without special precautions.

- 1 mL transfer pipettes (2 per student group)
- Q-tips or spreaders (1 per student group; spreaders can be washed and reused; possible source https://www.fishersci.com/shop/products/fisherbrand-l-shaped-cell-spreaders-2/p-4249846?xrefPartType=To&fromPartNum=50403863&toPartNum=14665231&xrefEvent=&savings=0.00)
- Spooling sticks to pull out DNA (preferably 1 per student in your largest class, but at least 1 per student group in your largest class; https://www.fishersci.com/shop/products/fisherbrand-plain-tipped-applicators-3/23400102?matchedCatNo=23400102; or you can use the blunt end of barbecue sticks)*
- Test tubes (1 for each student in your largest class; 12 x 75 mm or a little larger)*

*These will need to be washed and set out to dry for reuse in each class.

- Something to hold a test tube upright during the DNA extraction process (e.g. a test tube rack for each student group in your largest class; you may also want to have a tub to collect dirty test tubes)
- Chilled 70-95% isopropyl or ethyl alcohol (1 mL per student; you will probably want to have available a tub of ice, freezer, or refrigerator to keep the alcohol chilled and small beakers or jars to hold small amounts of alcohol for student use)

**Supplies and Preparation for Modeling DNA Replication**
- nucleotide pieces – A template for making enough nucleotide pieces for nine students or pairs of students is provided on the last page of these Teacher Preparation Notes. After you photocopy enough copies for the number of students you have, you can:
  - precut each page in nine parts and provide your students with scissors as well as tape or
  - recruit student helpers to precut each page to make 9 packets of 10 nucleotides each.
- tape

**Instructional Suggestions and Biology Background**

**General**

Before students begin the activity, they should have a basic understanding of the structure and function of proteins. A suggested sequence of learning activities for introducing students to proteins and DNA is provided in "Understanding the Functions of Proteins and DNA" (available at [http://serendip.brynmawr.edu/exchange/bioactivities/proteins](http://serendip.brynmawr.edu/exchange/bioactivities/proteins)).

We estimate that this activity will require 1½-2 50-minute periods, depending on your students and how much they know about DNA before beginning this activity.
In the Student Handout, numbers in bold indicate questions for the students to answer and ◗ indicates a step in the extraction or modeling procedures for the students to do.

If you use the Word version of the Student Handout to make changes for your students, please check the PDF version to make sure that the figures and formatting in the Word version are displaying correctly on your computer.

To help students understand the big picture and consolidate their understanding of DNA structure, function and replication, you may want to use a modified version of storyboarding with this activity, as follows:

- After students complete question 1 in the Student Handout, have students work in pairs to respond to the DNA Storyboard (shown on page 11 of these Teacher Preparation Notes). This will help to activate students’ memory of relevant concepts and information. We recommend that you review these initial storyboards to learn more about your students’ knowledge and any misconceptions they may have; this storyboard is intended for formative assessment only.
- As students increase their understanding of DNA during the activity, they can modify their storyboards.
- After completing the activity presented in the Student Handout, students complete the storyboard again without looking at their earlier storyboard or the Student Handout. After they complete the storyboards, students should have prompt feedback so they can improve the accuracy and completeness of their storyboards; you can accomplish this in a class discussion where students compare their storyboards. This type of active recall with feedback helps to consolidate student understanding and retention of the concepts learned during the activity.  

A key for the Student Handout and for the storyboard is available upon request to Ingrid Waldron (iwaldron@sas.upenn.edu). The following paragraphs provide additional instructional suggestions and background information – some for inclusion in your class discussions and some to provide you with relevant background that may be useful for your understanding and/or for responding to student questions.

You may want to briefly introduce students to archaea. (This group is sometimes known as Archaebacteria, but the term archaea is preferred to indicate that this group is a separate domain from bacteria and eukaryotes.)

Many Archaea are adapted to extreme environments such as very high salt concentrations. For example, Haloferax cells accumulate high concentrations of KCl to balance the high osmotic

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6 This general approach is described in "Using Storyboarding to Model Gene Expression", American Biology Teacher 77:452-457, 2015.

7 Evidence for the benefits of active recall with prompt feedback is described in http://www.scientificamerican.com/article/researchers-find-that-frequent-tests-can-boost-learning/.

8 http://www.zo.utexas.edu/faculty/sjasper/images/27T.2.gif
concentration of the extremely salty environments where *Haloferax* grows; many other halophiles accumulate high concentrations of relatively small organic molecules. Other Archaea live in a variety of environments such as the ocean, soil, and the human colon. A brief summary of the biology of Archaea and *Haloferax volcanii* is available at https://sites.google.com/site/molecularbiologyiw/home/haloferax-molecular-biology.

**Question 1** is intended to engage student interest and get the students thinking about what they already know about DNA.

**DNA Extraction**

Before your students begin the DNA extraction, we recommend that you show our [video](https://www.youtube.com/watch?v=Xc1ek1QKEU8&feature=youtu.be) which demonstrates the procedure, and/or you can personally demonstrate:

- how to use a pipette to add 5 mL of water on a plate of *Haloferax* and use a Q-tip or spreader to gently move all the lysed cells off the agar surface and mix the lysed cells with the water
- how to tilt the agar plate and use a pipette to suck up 1 mL of the solution with dissolved DNA to put in a test tube
- how to add alcohol.

As your students stir the lysed cells and water, they will be able to see the strands of DNA swirling in the mixture. (Because of these strands, the mixture will not be uniform.) The students should also be able to see the strands of DNA swirling as they suck the solution into their pipettes. In addition, the students should notice the increased viscosity of the solution; this increased viscosity is due to the very long DNA molecules dissolved in the water. You can use the figure on the bottom of page 1 of the Student Handout to emphasize how very long the DNA molecule is compared to the cell that contains it. You may also want to use the figures on the next page to help students visualize how long the DNA molecule is compared to a prokaryotic cell and/or to explain how super coiling of the DNA helps to pack the very long DNA molecule inside a tiny cell.

Each plate of *Haloferax* should have enough DNA solution to prepare a total of four test tubes. After the third test tube has been prepared from each plate, add an additional milliliter of water and stir again so the fourth student can pipette up 1 mL of solution with dissolved DNA for their test tube.

Cold alcohol helps to precipitate the DNA molecules by reducing the temperature and dehydrating the solution of DNA immediately under the alcohol layer. You may want to explain to your students how alcohol helps to precipitate the DNA. DNA is soluble in water because the negatively charged phosphate groups along the sugar phosphate backbone are attracted to the partial negative charge of the O atoms in the polar water molecules. Ethanol has a polar component, but also has a large nonpolar component, so DNA is less soluble in ethanol.
While the students are waiting for the DNA to precipitate, they should review the brief introduction to DNA structure and function on page 2 of the Student Handout and answer questions 3 and 4. It will be fine if the wait time after adding alcohol is longer than 20 minutes.

To ensure student understanding, the Student Handout includes multiple simplifications. For example, the definition of a gene on page 2 ignores multiple complexities, including the facts that many genes code for more than one polypeptide and many genes code for RNA that has different functions from mRNA.
Discussion of question 3 will provide the opportunity to reinforce student understanding that DNA carries the genetic information in all types of living organisms. You may also want to point out that the structure and function of DNA is similar in all types of organisms. Whereas question 3 discusses genes that are crucial for cell function and survival, question 4 refers to an example of a gene that is not crucial for cell function and survival, so a non-functional allele for this gene is not lethal and instead results in albinism.

The allele for albinism codes for a defective enzyme for producing melanin, a dark pigment in skin cells that protects their DNA from the damaging effects of the sun's UV radiation (similar to the protective function of the red pigment in *Halofexa*). In the most common form of albinism, the defective enzyme for producing melanin not only results in albino skin and hair color, but also affects the appearance and function of the eyes. You may want to point out to your students that skin color is also influenced by other genes (e.g. genes that influence how much melanin is made) and environmental factors (e.g. sun exposure which can result in increased production of melanin). Further information about albinism is available at [https://medlineplus.gov/ency/article/001479.htm](https://medlineplus.gov/ency/article/001479.htm) and [http://omim.org/entry/203100](http://omim.org/entry/203100).

After the 20-minute wait, when your students are ready to examine the extracted DNA, emphasize that they should first look at the undisturbed test tube; they should see a translucent layer where the DNA is located between the original mixture and alcohol, and they may see strands of DNA stretching up into the alcohol layer; sometimes the strands of DNA have bubbles on them. Tilt the test tube about 45°, put the stick provided ½ inch into the solution, and stir very gently in one direction only about 10 times. Then, slowly pull the stick up along the inside of the test tube; you will be able to see the trail of mucus-like DNA stretching behind the stick. Then, gently rub the stick on the edge of the test tube and stretch it outward to see the goopy, elastic strands of DNA.

**DNA Structure and Function**

In discussing the figure in the middle of page 3 of the Student Handout, you may want to remind your students that the solid lines represent covalent bonds and the dotted lines represent hydrogen bonds.
The boxed sentence near the top of page 4 of the Student Handout provides important background for helping students understand why **accurate replication of the sequence of nucleotides** in DNA is so important, as discussed further in question 10. You may want to show your students the following illustrated flowchart version of this information.9

```
sequence of nucleotides in the DNA of a gene

\[\text{determines the sequence of amino acids in a protein}\]

\[\text{determines the structure and function of the protein}\]

\(\text{(e.g. normal vs. defective enzyme to make skin pigment)}\)

\[\text{influences the characteristics or traits of the organism}\]

\(\text{(e.g. normal skin pigmentation vs. albino)}\)
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**Question 7** provides the opportunity to discuss the Crosscutting Concept, **Structure and Function**. Specifically, the structure of DNA contributes to its function as the molecule of heredity. The sequence of nucleotides in a gene codes for the sequence of amino acids in a protein which in turn determines the structure and function of the protein which influences the organism’s characteristics.

**DNA Replication**
The explanation of DNA replication on the bottom of page 4 provides another opportunity to discuss the Crosscutting Concept, **Structure and Function**.

For **questions 10-12**, after students have written their initial responses and you have had a class discussion of these responses, you may want to have your students prepare revised versions of their answers to one or more of these questions in order to consolidate accurate understanding. For question 11, if your students are not familiar with the use of the suffix "ase" to designate an enzyme, you will need to provide that information.

**Follow-Up Activities and Additional Resources** (NGSS is used to designate activities that are explicitly aligned with the **Next Generation Science Standards**.)

**UV, Mutations, and DNA Repair** (NGSS; [http://serendip.brynmawr.edu/science_edu/waldron/#UVmutations](http://serendip.brynmawr.edu/science_edu/waldron/#UVmutations))

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9 The image shown for the folded protein in this version of the flowchart differs from the image of the protein on page 2 of the Student Handout. The image shown on page 2 is more accurate ([http://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/enzymes/GetPage.pl?ec_number=1.14.18.1](http://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/enzymes/GetPage.pl?ec_number=1.14.18.1)); the second and third images in the flowchart are designed to suggest how a polypeptide folds into a functional protein.
In this activity, students learn about the effects of UV light, mutations and DNA repair on the survival of prokaryotes and the risk of skin cancer. In the first experiment, students evaluate the effects of different durations of UV exposure on survival and population growth of *Haloferax volcanii*. This experiment also tests for photorepair of DNA damage. Students design the second experiment, which evaluates the effectiveness of sunscreen. In addition, students answer analysis and discussion questions that promote their understanding of molecular biology, cancer, and the interpretation of experimental results. (NGSS)

To further develop student understanding of how DNA provides the instructions for protein synthesis and influences our characteristics, we recommend:
– our analysis and discussion activity From Gene to Protein via Transcription and Translation (NGSS; http://serendip.brynmawr.edu/exchange/bioactivities/trans) or
– our hands-on modeling activity From Gene to Protein – Transcription and Translation (NGSS; http://serendip.brynmawr.edu/sci_edu/waldron/#trans).

To help students understand how chromosomes are separated during cell division and how genes are transmitted from parents to offspring, we recommend our hands-on modeling activities:
– Mitosis - How Each New Cell Gets a Complete Set of Genes (NGSS; http://serendip.brynmawr.edu/sci_edu/waldron/#mitosis) and
– Meiosis and Fertilization – Understanding How Genes Are Inherited (NGSS; http://serendip.brynmawr.edu/sci_edu/waldron/#meiosis).

To ensure student understanding of the basics of DNA structure, function, and replication, this activity ignores many complexities. For additional information, see:
– helpful resources available at http://learn.genetics.utah.edu/content/molecules/ and http://www.hhmi.org/biointeractive/teacher-guide-dna
– a college textbook for biology majors such as Campbell, Reece, et al., *Biology*; Freeman et al., *Biological Science*; or Raven et al., *Biology*

One important point that is not included in the Student Handout is that, during actual DNA replication, sometimes mistakes are made and the wrong nucleotide is added to the new strand of DNA. DNA polymerase can “proofread” each new double helix DNA strand for mistakes and backtrack to fix any mistakes it finds. To fix a mistake, DNA polymerase removes the incorrectly paired nucleotide and replaces it with the correct one. If a mistake is made and not found, the mistake can become permanent. Then, any daughter cells will have this same change in the DNA molecule. These changes are called point mutations because they change the genetic code at one point, i.e. one nucleotide. A point mutation in a gene in a gamete that forms a zygote can result in significant effects, such as sickle cell anemia. (See e.g. The Molecular Biology of Mutations and Muscular Dystrophy (NGSS; http://serendip.brynmawr.edu/exchange/bioactivities/mutation/).)

Additional background information and suggestions for follow-up activities are provided in:
– Molecular Biology: Major Concepts and Learning Activities (http://serendip.brynmawr.edu/exchange/bioactivities/MolBio)
1. Write sentences and label the figure to describe the structure of DNA.

2. Complete this table to describe how two different versions of a gene can result in normal skin and hair color vs. albinism.

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="DNA double helix" /></td>
<td><img src="image" alt="molecule" /></td>
<td><img src="image" alt="people" /></td>
</tr>
<tr>
<td><img src="image" alt="DNA double helix" /></td>
<td><img src="image" alt="molecule" /></td>
<td></td>
</tr>
<tr>
<td><img src="image" alt="DNA double helix" /></td>
<td></td>
<td>Normal skin and hair color</td>
</tr>
<tr>
<td><img src="image" alt="DNA double helix" /></td>
<td></td>
<td>Albinism (very pale skin and hair)</td>
</tr>
</tbody>
</table>

3. Describe how DNA is replicated.
Nucleotides for Nine Students or Pairs of Students