DNA and Identity!

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About DNA

Deoxyribonucleic Acid (DNA) is widely known as the blueprint of life. DNA exists as a pair of polymer chains; each chain consists of a structural "backbone" of sugars and phosphate groups with **nucleotide bases** attached to each sugar. There are four bases occuring in DNA: Adenine, Cytosine, Guanine, and Thymine. Unless it is being transcribed or replicated, DNA exists in a spiraling arrangement called a **double-helix**.

In this arrangement each base on one strand pairs with its complementary base on the second strand (Adenine pairs with Thymine and Cytosine pairs with Guanine). It is the specific order of these bases along the DNA molecule that actually represents all of the instructions for creating a living organism.

The information "written" in DNA is actually limited to recipes for the creation of chemicals; specifically, DNA contains the instructions for making all of the **proteins** needed by an organism to survive.

Proteins are chains of components called **amino acids**. The arrangement of these amino acids and their binding affinity for each other cause the resulting molecule to form unique shapes, which are crucial for the proteins' functions.

Many proteins, such as **enzymes**, serve as **catalysts** for an amazing variety of chemical reactions that organisms depend upon to remain alive. Other proteins serve as structural pieces of cells that allow for regulation of different substances to pass through cell membranes. Still others, such as **hormones**, are created and sequestered in cells to be released when cells receive signals from the nervous system. While DNA itself does not instruct the cells or the body to perform specific tasks, all of an organism's cells and systems depend on the interaction of proteins to accomplish all vital functions.

In simple organisms such as bacteria (**prokaryotes**), DNA is free-floating in the cytoplasm of each cell. In protists, fungi, plants, and animals (**eukaryotes**), DNA is sequestered in a large organelle inside each cell – the nucleus.

While most organisms have DNA in every cell, there is an important exception: mammalian red blood cells do not contain a nucleus and cannot reproduce themselves or create new proteins. Each cell contains not one but two complete copies of an organism's DNA. This is one of the ways organisms have developed to limit the effects of DNA damage, called **mutations**, on organisms and their offspring.

Most of the time, DNA exists in the nucleus in uncoiled strands called **chromatin**. It is only when a cell is ready to divide that the chromatin twists itself into sausage-shaped **chromosomes**. Before division (**mitosis**), a cell replicates its DNA so that there are four copies of each chromosome.

Extraction (or isolation) of DNA requires simple but important steps. First, cells must be released from tissues. DNA is held within the nuclear membrane, which is inside the cell membrane. Both of these membranes must be **lysed** (broken open) in order to remove the DNA. Enzymes that exist within the cell (but not within the nucleus) have the capacity to degrade DNA, and other proteins and carbohydrates tend to bind to the released DNA, reducing its purity. Even after these other materials have been controlled for, DNA must be removed from a mixture.

Releasing cells from tissues usually involves crushing or blending. The resulting slurry, called the **homogenate**, is then passed through a filter to remove large pieces of tissue.

MOST*

VOCABULARY

Amino Acids
Catalysts
Chromatin
Chromosomes
Diploid
DNA
Double-helix
Electrophoresis
Enzymes
Eukaryotes
Homogenate
Hormones
Lysed
Meosis
Mitosis
Mutations
Nucleotide Base
Octoploid
Prokaryotes

Inside This Packet

New York State Standards

Living Science High School Level

Standard 1:
Key Idea 1: 1.4a
Key Idea 2: 2.3a, 2.3b

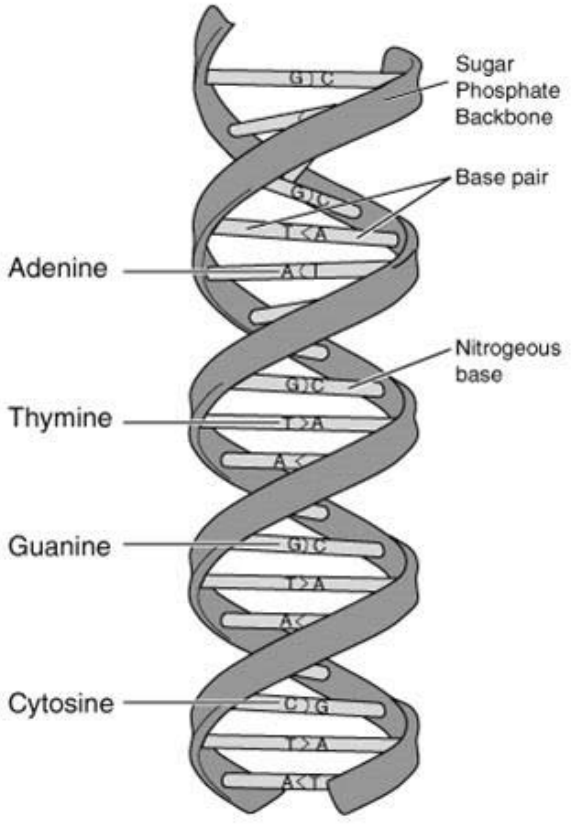
Standard 4:
Key Idea 2:2.1a, 2.1b, 2.1c, 2.1e, 2.1f, 2.1g, 2.1h, 2.1i

Membranes, being composed of phospholipids, can be broken easily with the use of detergents. The DNA can also be protected from effects of other cell chemicals by addition of salt (NaCl). When the salt dissociates into Na+ and Cl- ions, these charged particles prevent the negative charge of the DNA from binding to the positive charges on proteins.

Finally, we can use DNA's solubility to pull it out of solution. Using nearly pure, chilled alcohol poured into a layer above the soap-salt-homogenate mixture, DNA can be pulled gently into the alcohol layer and spooled around a wire or toothpick.

DNA can be extracted from any kind of cell. Strawberries provide an extremely good source, however, because of their elevated ploidy level. Most organisms are **diploid**, meaning they have two sets of chromosomes. Some plants and animals exist with more than two complete copies of their DNA in each cell, and are called **polyploid**. Strawberries have eight complete sets of chromosomes, and are known as **octoploid**. For extraction purposes, this means four times as much DNA can be obtained from strawberries than from an equal number of cells of diploid tissue.

There are advantages and disadvantages to polyploidy. Plants that are polyploidy may be able to produce larger fruit faster, and may be shielded somewhat from effects of deleterious mutations. On the other hand, these extra copies of chromosomes take up a great deal of extra volume in each cell, and increase the likelihood of aberrations during mitosis and meiosis.



Activity: Strawberry DNA

MATERIALS NEEDED

1 large strawberry OR 2 small strawberries
1 small sealable freezer bag
10 mL detergent solution
graduated cylinder
1 small funnel
2-3 layers cheesecloth (or a coffee filter)
1 50 mL beaker or similar container
1 dissecting pin/inoculating loop/ bamboo skewer
2 test tubes
1 rubber band
cold alcohol (91% concentration)
disposable pipets

Students should be able to:

Extract DNA from a strawberry.

Define the terms describing DNA and the extraction process.

DNA is an important chemical responsible for storing all of the information a cell needs to create proteins. It is housed inside the nucleus of eukaryotic (plant, animal, fungus, and protist) cells.

DNA can be removed from cells and collected using a process called DNA extraction. The process must first remove DNA from inside cell and nuclear membranes. Once these membranes are destroyed, however, DNA tends to bind to proteins that are also freed and floating in the mixture. A solution of detergent and salt helps to break the membranes and neutralize the charges on DNA and proteins, preventing them from binding together. Finally to see the DNA, it can be pulled from solution using alcohol. This is because DNA is insoluble in alcohol. After DNA is extracted, researchers can run tests such as electrophoresis (DNA fingerprinting) or sequencing (to determine the sequence of nucleotide bases in the extracted DNA).

In this activity, you will extract a sample of DNA from strawberries. Strawberries are an easy subject to use since they are easily obtained and processed and because they have multiple copies of their DNA in each cell. Humans have two copies of DNA in each cell (except gametes and red blood cells), and are called diploid. Strawberries contain four times as much DNA, they have eight copies of DNA in each cell and are called octoploid.

Step 1:
Collect the necessary materials from the supply table and then read through the entire procedure before beginning the activity.

Step 2:
Place your strawberry inside the bag and try to seal it without much air inside. Place the bag flat on your table with the strawberry in the center. Mash the strawberry with your fingers, taking care not to break the bag.

Step 3:
Bring your bag to the supply table. Carefully measure 10 mL of detergent solution and pour it into your bag. Return to your table.

Step 4:
Gently mix the mashed strawberry and detergent solution. Try not to generate too many suds, mix for 2 to 3 minutes.

Step 5:
Place the damp or wet filter cloth (or coffee filter) inside the beaker or container and place the rubber band around the top to hold the cloth in place. Slowly pour the contents of your bag into cloth. Do not pour more than can fit inside the cloth, allow some liquid to filter if there is not enough room.

Step 6:
Allow the liquid to filter through for around 5 minutes. Remove the cloth and place it inside your bag. Carefully pour the filtered solution into your test tube until it is around half-full.

Activity: Strawberry DNA

Name: _____ *Date:* _____

Step 7:
Bring your test tube to the supply table and gently pipette some chilled alcohol into the test tube. You should hold the test tube at an angle and slowly drip the alcohol down the side of the tube. The alcohol should float on top of the strawberry-detergent solution. Take note that this alcohol is toxic.

Step 8:
Return to your table and carefully dip your wire loop/dissecting needle/skewer into the alcohol, then slightly into the strawberry solution. It should not be pushed too deep into the strawberry solution. Gently pull it up into the alcohol and twist it slowly. Continue to dip and twist into the alcohol until you can see a whitish mass developing on the tip of the wire loop/dissecting needle/skewer. This is the DNA.

Step 9:
Continue spooling your DNA until you feel you have collected all of it. Gently pull it from the test tube and place it into your clean test tube. Clean up your station and the equipment you used. Compare the DNA you extracted with other students, and begin the activity questions.

Activity Questions:

Try to answer these questions, when everyone is finished with the extraction, your instructor will lead a discussion to answer them with you.

1. Describe what is happening at a cellular level at each of the following steps: Physical mashing of the strawberry, mixing of strawberry and detergent-salt solution, pulling of wire loop/dissecting needle/skewer through the alcohol layer.

2. If we were to compare strawberry fruit and human epithelial cells, and these samples contained exactly the same number of cells, how would the quantity of DNA differ between them?

3. Describe two different real-world applications of DNA extraction.

Activity Prep

Have supplies arranged on a table so students can gather their materials. All solutions should be kept as cold as possible, have these on ice. Instruct students to take the alcohol last (it will be easiest to have students pipette it into their test tubes when they need it instead of collecting it in a separate tube).

Activity Introduction:
Begin by engaging students in a refresher discussion on DNA. Make sure to recall where the DNA is found and what it does. Describe the process briefly, making sure to note what steps break the cell membrane, and what parts of the process help remove other cell materials from the DNA. Finally, discuss the idea of solubility and how DNA precipitates out of solution in alcohol.

Pass out the Student Activity Sheet for this activity and ask students to read the background and methods; when they are done they may begin by collecting their equipment and materials.

Running the Activity:
Make sure students do not mash the strawberry fervently enough to break the bag.

Warn students not to mix the alcohol and the processed strawberry liquid by jetting the alcohol into the test tube. Instruct them to hold the test tube at an angle and gently squeeze the pipette. The alcohol should stay in a discrete layer on top of the strawberry liquid.

Make sure students know that the alcohol used in this experiment, be it research-grade ethanol or methanol, is not the edible kind and is toxic if ingested. Wear safety glasses while working with alcohol.

Once students are finished, they may begin working on the activity questions. These questions call for some extra knowledge and would be best answered in a class discussion.

Activity Materials:
1 large strawberry OR 2 small strawberries per student/group
1 small sealable freezer bag per student/group
10 mL detergent solution (see solution prep notes) per student/group
graduated cylinder to measure detergent solution
1 small funnel per student/group (helpful, but not essential)
2-3 layers cheesecloth (or a coffee filter), cut to fit funnel per student/group
1 50 mL beaker or similar container per student/group
1 dissecting pin/inoculating loop/bamboo skewer per student/group
2 test tubes per student/group
1 rubber band per student/group
alcohol (91% concentration) on ice
disposable pipets

Solution Prep Notes:
Detergent solution
Mix gently 900 mL water, 50 mL dishwashing detergent, 2 tsp salt
(you may choose to have students add the ingredients directly to their crushed strawberry. In this case, have students add 2 tsp of water, 1 tsp detergent, and a pinch of salt).

Source Material

Carolyn A. Zanta, UIUC-Hughes Biotechnology Education & Outreach Program: www.life.uiuc.edu/hughes/footlocker