Strawberry-DNA Extraction

Workshop 86 Bio

Preparation Before Class

- Get the number of Strawberry-DNA Extraction kits needed for class, the teacher demo box, and the teacher-prep box.
- Set up the teacher table with a DNA model, a poster, and extra materials.
- Lay down bench liners on desks.
- Place Strawberry-DNA Extraction kits on tables.

Objective

- After first making a buffer solution, students will extract and isolate DNA from a strawberry by following common DNA-spooling techniques.
- Students will be able to explain how a buffer solution disrupts the cell's plasma membrane, releasing cellular components into the solution.
- This experiment will provide a hands-on activity and an opportunity for students to develop an appreciation for the physical nature of DNA and the process of DNA purification.

Concepts

- Students will learn about the various parts of the DNA molecule and what *helical* means. By using a model, we hope to better allow students—especially visual learners—to visualize the molecule and its structure.
- You'll introduce the names of the bases—adenosine, thymine, guanine, and cytosine—and explain how they pair up with one another. This introduction will provide

students with basic knowledge about DNA that will be important for future lessons.

- You'll explain the importance of the deoxyribose background in the structure of DNA.
- By learning that DNA can be separated, students will gain a foundation of knowledge for future lessons in biology, evolution, biotechnology, and health technology.

California State Science Standards Connections

Second grade: Life Sciences: 2a, c; Investigation and Experimentation: 4a-d, g

Third grade: Investigation and Experimentation: 5a-e

Fourth grade: Investigation and Experimentation: 6a-f

Fifth grade: Physical Sciences: 1e-f, h; Life Sciences: 2a; Investigation and Experimentation: 6b-i

Sixth grade: Investigation and Experimentation: 7a-h

Seventh grade: Cell Biology: 1c; Genetics: 2b-e; Investigation and Experimentation: 7a-e

Eighth grade: Chemistry of Living Systems (Life Sciences): 6a-c; Density and Buoyancy: 8a-d; Investigation and Experimentation: 9a-c

Teacher Background

DNA molecules are long, slender molecules that carry the heritable information of organisms on to future generations. Because of its microscopic size, it is impossible to see a DNA molecule with the naked eye. It would take about 300,000 DNA molecules side by side to make a bundle as thick as a human hair. When subjected to certain conditions, it is possible to collect "large" amounts of DNA to make it visible.

This process of collecting DNA is referred to as spooling. During spooling, you mix a

buffer solution, made from soap, with the cell. The soap from the buffer solution disrupts the cell membrane's phospholipid bilayer by reacting with the phosphate group of the phospholipid. This action releases the cell's components into the buffer.

After you've mixed the buffer with the DNA source, you carefully overlay the buffer/DNA solution with isopropanol (rubbing alcohol); RNA and DNA precipitate in solutions containing high percentages of isopropanol or ethanol. Due to its size and abundance, chromosomal DNA forms viscous, clotted masses during such alcohol precipitation. You can use a plastic loop to mix the two liquids at their interface and to collect the DNA as it precipitates in the solution at the mixing zone.

Small fragments of DNA and degraded RNA usually contaminate the chromosomal DNA during extraction procedures. The alcohol also precipitates these fragments, but they have little tendency to spool on the loop because they are too short and form finer, more uniform precipitates. Therefore, you can view spooling as a method that partially purifies and concentrates high-molecular-weight DNA.

The purification of chromosomal DNA is frequently the first step in molecular-cloning experiments. Scientists can collect and redissolve the precipitate in a smaller volume. This is a convenient way to concentrate nucleic acids. Alcohol precipitations also remove small molecules, such as buffer salts, sugars, and amino acids, from nucleic acid precipitations because they remain in the solution.

Getting Connected: What Do the Students Know? (five minutes)

Ask your students:

- What is DNA?
- Is it found in all cells?
- How long is the DNA in a single cell?

Tell the students, “All living organisms are composed of cells. Organisms, such as bacteria, are single cells, while very complex organisms, such as humans, are composed of billions of many different kinds of cells. Human cells contain a nucleus, which contains forty-six chromosomes in twenty-three pairs. Chromosomes contain DNA, which encodes all the genetic information that is inherited from parents.”

Unwind the DNA model on the teacher table and say to the class, “James Watson and Francis Crick determined the structure of the DNA molecule in 1953. They figured out that DNA is a double helix consisting of two strands. The Watson-Crick model is often described as a spiral ladder.

“DNA is made up of building blocks known as nucleotides. Each nucleotide is composed of three parts—a phosphate group, deoxyribose sugar, and one of the four nitrogenous bases: adenine, guanine, cytosine, or thymine. The two strands of DNA are the backbone of the ladder, made of carbohydrate sugar phosphodiester groups. The sugar backbone acts as a support for the rungs of the ladder. The rungs are composed of the nitrogenous bases. Scientists use the first letters of these bases—A, G, C, and T—to designate the order of the bases within the DNA strands. The bases are always arranged in pairs. When A occurs on one strand, T will occur on the opposite strand. Similarly, G and C are base pairs on opposite DNA strands. The bases are held together by weak hydrogen bonds.

“DNA plays an important role in two processes. During the process of replication, DNA provides information to copy itself, so genetic information can be passed on from one generation of cells to the next. DNA also provides instructions for making proteins, and these instructions are vital to the maintenance and function of cells. DNA provides the information on how to order the amino acids required for making various proteins.”

Making It Happen

Safety Note: Students must wear blue nitrile gloves and goggles as standard laboratory practice.

Materials

a pocket scale

gloves and goggles

liquid dish soap

distilled water

50-ml and 100-ml beakers

ice-cold 99 percent isopropanol (rubbing alcohol)

a strawberry

a quart-size, zippered plastic storage bag

a funnel

a paint filter

a test tube

a cocktail straw

Notes on Materials

The plastic bag should be as thick as possible. Those designed for freezer storage are thicker and resist breaking much better than the sandwich type.

Strawberries can be fresh or frozen. If using frozen strawberries, thaw them out before the lab. Other soft fruits, such as kiwis or bananas, will work, but they do not yield nearly as much DNA.

Preparation of DNA-Extraction Buffer (enough for a hundred groups)

Materials:

100 ml (3/8 cup) of shampoo (without conditioner; 50 ml of liquid dish soap can be substituted)

15 g (2 tsp.) NaCl (sodium chloride, or salt)

900 ml of water

Isopropanol must be at least 90 percent, and it needs to be cold. Putting it in several small dropper bottles that you keep on ice in the front of the room makes it easy to dispense.

Ask the students: "How do you extract DNA from something? Well, first, you need to find something that contains DNA. Because DNA is the blueprint for life, everything living contains DNA. For our workshop, we're going to use strawberries. Of course, there are many other DNA sources we could use, such as spinach, broccoli, bananas, or chicken liver. And there are sources of DNA that we shouldn't try to use, such as a family pet, your little sister, or the little critters you find in the backyard.

"We picked strawberries because they are soft and easy to mash. Also, ripe strawberries

contain special enzymes called pectinases and cellulases, which help break down the cell walls."

After this introduction, ask the students, "What is an enzyme, anyway?" (Wait for their answers.)

"Enzymes are proteins that help chemical reactions happen more quickly. Without enzymes, our bodies would grind to a halt. Our strawberry enzymes cut proteins just like a pair of scissors.

"Finally, we picked strawberries because a strawberry has an enormous genome, a lot of genetic material. Strawberries have eight of each type of chromosome, so they are called octoploid."

Procedure

- Use a marker to indicate 10 ml on a graduated cylinder. Add 10 ml of extraction buffer.
- Put a paint filter in the funnel, then put the funnel on top of a test tube.
- Place the isopropanol on ice.
- Weigh a strawberry. Record the weight.
- Put the strawberry in a plastic storage bag and zip closed. Squeeze as much air out of the bag as possible.
- Smash the strawberry as much as you can for one to two minutes.

Tell the students, "Mashing separates the strawberry cells from one another, so you now

have a really thin strawberry-cell soup."

- Open the bag, add the 10 ml of extraction buffer, and zip the bag closed while getting out as much air as possible.
- Mix the strawberry with the extraction buffer, using your fingers on the outside of the bag, for one minute.

Ask the students, "Why am I adding the extraction buffer, the detergent, to the strawberry-cell soup?" (Wait for answers.)

Tell your students, "Blending separated the strawberry cells. But each cell is surrounded by a sack, the cell membrane. DNA is found inside a second sack, the nucleus, within each cell. To see the DNA, we have to break open these two sacks. We do this with the detergent in our extraction buffer. But how does the detergent work?

"Think about why you use soap to wash dishes or your hands. You use it to remove grease and dirt, right? Soap molecules and grease molecules are made of two parts—they have heads, which like water, and they have tails, which hate water.

"Both soap and grease molecules organize themselves in bubbles, spheres, with their heads outside to face the water. When soap comes into contact with grease, they combine because they have similar structures, forming a greasy, soapy ball.

"The membranes in a cell have two layers of lipid, or fat, molecules with proteins going through them. When detergent comes close to the cell, it captures the lipids and proteins.

After adding the detergent, what do you have in your strawberry soup?"

- Use scissors to cut a small corner off of the plastic bag.
- Squeeze the strawberry mush into the paint filter fitted into the funnel and let it drip into the straw until it is about 1/4 to 1/3 full.
- Gently add 10 ml of ice-cold isopropanol to the mixture in the test tube by tilting the tube and allowing alcohol to dribble down the side.

Ask the students, "Why are we adding the alcohol?" Then, tell them, "I have a hypothesis. Alcohol is less dense than water, so it floats on top. Because two separate layers are formed, the grease and the protein that we broke up in the first two steps, and the DNA, all have to decide, 'Hmm, which layer should I go to?' This is sort of like looking around a room for the most comfortable seat. Some will choose the couch, but others might choose the rocking chair.

"In this case, the protein and grease parts find the bottom, watery layer to be the most comfortable place, while the DNA prefers the top, alcohol layer. Where the two layers meet is called an interface.

"So, what is that stringy stuff in your tube? That is strawberry DNA!"

- Insert the cocktail straw into the test tube. Carefully swirl the straw just below the interface. Wind (spool) the DNA that comes out of the solution onto the loop. (Keep in mind that these are not single DNA molecules, but thousands of molecules.) After a minute of spooling, slowly remove the rod from the tube. The DNA—a clear, viscous,

clotted mass—will adhere to the rod. Examine and touch the DNA on the loop.

Tell the students, "Now that we've successfully extracted DNA from one source, we are ready to experiment further. We are going to have each team try one of these experiments:

Team 1: Repeat the DNA-extraction procedures with other fruits, making sure the fruits are the same weight as the strawberry. Please be sure to record which fruit gives you the most DNA. How can you compare them?

Team 2: Experiment with different soaps and detergents. Do powdered soaps work as well as liquid detergents? How about shampoo or body scrub?

Team 3: Experiment with leaving out or changing steps. We've told you that you need each step, but is this true? Find out for yourself. Try leaving out a step or changing how much of each ingredient you use.

Team 4: Do only living organisms contain DNA? Try extracting DNA from things that you think might not have DNA.

Wrapping It Up: What Did the Students Learn?

Student Evaluation -- Questions to Encourage Teaching Points

- What did the DNA look like? Relate its chemical structure to how it looks when a lot of it is clumped together.

(The DNA looked like spider webs. The DNA precipitate looked like long and thin fibers. This makes sense because the molecular structure is so long and narrow.)

- DNA is soluble in water, but not in isopropanol or ethanol. What does this fact have to do with our method of extraction? Explain what happened when the isopropanol came in contact with the strawberry extract.

(The DNA was soluble in the DNA extraction buffer, so we could not see it. When it got stirred into the isopropanol, it clumped together and formed thicker and thicker strands that were large enough for us to see.)

- A person cannot see a single cotton thread from 100feet away but would be able to see at some distance thousands of threads woven together into a rope. How is this analogous to what we experienced with our DNA extraction?

(DNA is far too narrow to see, but if there are many thousands of strands clumped together, it is thick enough to be visible.)

- In order to study our genes, scientists must first extract the DNA from human tissue. Would you expect the method of DNA extraction to be the same for human DNA as it was for strawberry DNA? Why or why not?

(Unlike plant cells, animal cells do not have cell walls. Therefore, it isn't necessary to filter out the cellulose debris from an animal cell. Also, animal cells can be lysed, or disintegrated, if put in a hypotonic, or lower-pressure, solution.)

- Would the DNA be the same in every cell in the human body?

(Yes, because everyone starts off as one cell, which grows into an organism by undergoing mitosis. Therefore, all of the DNA in our cells is identical.)

- If you wanted to extract DNA from a living person, which cells would you use, and why?

(Blood is the easiest tissue to obtain from a living person. Although a red blood cell does not contain a nucleus, a white blood cells does. Skin cells will also work if you need only a small amount of DNA.)

- List two reasons why a scientist might want to study the DNA of strawberries.

(Scientists may want to
—examine the DNA of a certain type of strawberry that is more disease or frost resistant than other strawberries.
—study the evolutionary relatedness of strawberries to other berries.
—study a gene that codes for a particular protein in strawberries. For example, strawberries are known to have chemicals in them that slow the growth of some tumors.
—clone a particular gene in strawberries. Perhaps they want to make large quantities of the protein that makes strawberries red or that produces their flavor.)

Cleanup

Have children dump trash in white buckets and place items that are not trash back into the kits.

For more information, go to the Access Excellence Activities Exchange and view "Investigating DNA" (www.accessexcellence.org/AE/AEC/CC/investigating_DNA.html).