Microbial Discovery Activity

Taking the Mystery Out of DNA: Extracting DNA from Strawberries

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Intended Audience
K-4
5-8  X
9-12  X

Activity Specifications
Classroom setting   X
Requires special equipment   X
Uses hands-on manipulatives   X
Requires mathematical skills
Can be performed individually   X
Requires group work
Requires more than one (45 min) class period
Appropriate for special needs student   X
Introduction

Description
In this activity, students will extract DNA from living cells.

Abstract
Students will explore how scientists isolate deoxyribonucleic acid, DNA, from the strawberry. The activity provides the students with a hands-on approach to DNA isolation. They will learn how cells can be broken open and how the DNA can be separated from the rest of the cellular biological molecules. Although students may recognize DNA as being the genetic material and that it can be used in forensics to identify a killer, most are not exposed to what DNA looks like. This activity brings DNA to life!

Core Themes Addressed

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Keywords
Biotechnology, recombinant DNA technology, genetic engineering, deoxyribonucleic Acid (DNA), plant cells, extraction, precipitate, hydrogen bonding.

Learning Objectives
At the completion of this activity, the students will be able to:
• To explain the difference between a prokaryotic and eukaryotic cells.
• To explain some differences in cellular structure between animal cells and plant cells.
• To explain the structure of deoxyribonucleic acid, DNA.
• To be able to explain and then carry out the breakage of cells to release cellular constituents.
• To explain and then carry out the separation of DNA from other cellular components.

National Science Education Standards Addressed

Standard A: Science as Inquiry- In completion of this activity, students will investigate cell structure and the methods to extract DNA.

Standard B: Physical Science- In completion of this activity, students must consider the basic chemistry of the DNA molecule and how that relates to the DNA extraction processes. In particular, students will learn about hydrogen bonding, viscosity, dehydration and polymer properties.

Standard C: Life Sciences- In completion of this activity, students will examine eukaryotic cell structure and investigate the function and the structure of DNA in living organisms. Students explore DNA as being the molecular basis of heredity

Standard E: In completion of this activity, students will identify one of the key steps of the genetic revolution by isolating DNA.
Student Prior Knowledge
Students will need a general understanding of the structure of DNA and cellular structure.

Background

**Biotechnology & Applications:**
Biotechnology is the use and manipulation of living organisms to provide a practical outcome or product that should benefit mankind. Biotechnology is a broad term that may include different laboratory procedures and applications. Modern biotechnology often involves the manipulation of the deoxyribonucleic acid, DNA from living organisms. These applications that involved the manipulations of DNA is referred to as recombinant DNA technology or genetic engineering. Recombinant DNA technology generally involves the isolation of the DNA or genes, the alteration of genes, and the ability to move genes from one organism to another.

What are some practical applications of biotechnology? Recombinant DNA technology allows for the production of medicine. One example is human insulin that is used to treat diabetes. The human insulin gene can be isolated from the human chromosome and then inserted into a bacterium. The bacteria will produce the insulin protein that can then be isolated and purified and ultimately used as a drug to treat diabetics.

Recombinant DNA technology may be used to study and treat human diseases. Scientists are working to identify the genes that are responsible for genetic diseases. Individual with a particular disorder may have a mutation or change in their DNA leading to the disease. **Gene Therapy** is a modern approach to fix the mutant genes inside the human. Cells from the patient are isolated. These cells are manipulated so that they take in the normal gene and the normal gene will replace the faulty gene. The genetically engineered cells are placed back into the patient. The patient will now carry cells with the corrected gene.

Genetic engineering is used in agriculture to hopefully improve the productivity of crops. Plants can be genetically engineered to carry genes that result in resistance to insects or improved growth characteristics. The use of recombinant DNA technology is integrated in many aspects of our everyday lives even though we do not always recognize it. Even the vegetables that you eat may be genetically modified foods.

In order to perform recombinant DNA technology or simply to study genes, one must isolate the DNA from cells. In this exercise, your students will explore how DNA can be isolated from cells by a process referred to as a **DNA extraction**.
DNA Extraction from Strawberries:
When a scientist needs a source of DNA, it must be extracted from the cells that are being studied. In this exercise students will extract DNA from strawberries. This exercise allows the students to visualize the chromosomal DNA thereby reinforcing the concepts learned about DNA structure.

The chromosomal DNA is the blueprint for an organism determining what that organism will look like. DNA contains all the genetic information that forms the basis of inheritance and directs the making of proteins. The strawberry is a eukaryotic plant cell containing the chromosomal DNA inside the nucleus. In order for one to be able to extract and visualize the DNA, one must have a large quantity of DNA inside the test tube. This means that the DNA must be extracted from a large number of cells. A single strawberry contains many cells providing a good cellular sample for DNA. The strawberry is octoploid meaning that it contains eight copies of each type of chromosome and this provides a large amount of DNA that can be isolated from one or two strawberries.

What are the basic steps for extracting DNA from eukaryotic cells such as a plant cell? The entire process can be divided into four basic steps. There are special procedures and reagents that are used to accomplish each of these steps. The four steps include: disrupting the cell wall, lysing the cytoplasmic membrane (also called the plasma membrane), lysing the nuclear envelope, and separating out the DNA from the rest of the cellular material.

First the cell wall and cytoplasmic membrane surrounding the plant cell must be disrupted or broken open. The process of “breaking open” the cell is referred to as “lysing the cell”. Once the cytoplasmic membrane is lysed the nucleus is released. Since the nuclear envelope surrounds the DNA, this membrane must also be broken open to completely release the DNA. Lysing the plasma membrane and the nuclear envelope can be accomplished together since both are membranes with a similar composition of lipids and proteins. Detergents are useful reagents that assist with disrupting the membranes. The molecules that make up the detergent interact with the lipids and proteins and act to pull them away from the membrane. Therefore one can easily disrupt membrane structure to lyse the cytoplasmic membrane and nuclear envelope by manually squashing the strawberry and adding a detergent.

The strawberry is a plant cell and therefore like other plant cells, the strawberry cell contains a cell wall composed of cellulose located outside of the plasma membrane. The cell wall can sometimes be broken by physical considerations; some people freeze their plant tissue and then mash with a mortar and pestle. However, one benefit of using a ripened strawberry is that specialized steps to break down the cell wall are unnecessary. As a strawberry ripens it produces pectinases and cellulases, enzymes that break down the cell wall. The ripened fruit does the work of breaking down the cell wall structure making it easier for you to break open the cells and extract the chromosomal DNA.

Following the lysing of the plasma and nuclear membranes, a cell lysate (extract) has been generated which contains the released DNA along with all the other cellular components. The next step is to separate out the DNA from all of the other molecules of the cell. The DNA is precipitated and thereby separated by using ethanol and salt. Recall that the DNA molecule is double stranded and each strand is composed of a phosphate-sugar backbone (the sides of the ladder) with the nitrogen containing bases that hydrogen bond with the bases from the other strand (the rungs). The outer phosphate groups on the phosphate backbone provide negative charges that can hydrogen bond with water molecules. The ability of DNA to hydrogen bond to water molecules allows DNA to be soluble in water. Ethanol carries a hydroxyl group that is capable of forming hydrogen bonds with water. When ethanol is added to the cell lysate, the ethanol forms hydrogen bonds with water molecules and pulls the water away from the DNA. The DNA is insoluble in ethanol. The negative charges in the DNA will interact with the charges from the salt. Since the DNA can no longer interact with water molecules, it aggregates with other DNA molecules and salt thereby precipitating or separating out from the rest of the cellular components. Following the addition of the ethanol and salt, the DNA precipitates and appears as a stringy white substance.
Class Time
Approximately 45 minutes of class time are required.

Teacher Preparation Time
Teacher preparation time will vary depending on approach. Generally, less than one hour of preparation time is required in order to acquire the strawberries and to set up the materials for the students. Strawberries and ethanol should be refrigerated prior to class.

Materials and Equipment
- Frozen or fresh strawberries
- Plastic baggie: Use a heavy-duty zip lock baggie. The freezer storage zip lock bags work best because they will not tear as easily.
- 1 test tube (or small beaker) per extraction
- 1 test tube rack (is a test tube is being used) per extraction or shared
- 1 funnel per extraction
- 1 small piece of cheesecloth per extraction
- 10 ml of DNA extraction buffer per extraction
- a glass cylinder or 10 ml pipette to measure out the DNA extraction buffer.
- Ethanol in a squirt bottle (keep on ice).
- 1 Glass rod or wooden stick per extraction

Instructor Preparation for the Activity:
1. The instructor may allow the students to obtain their own strawberry and plastic bag. However, if time constraints in class are present, the instructor can place 1-2 strawberries in a plastic bag for each student in advance. These can be kept in the freezer until the day of class. Thaw before using!

2. Prepare the DNA extraction buffer. 1000 ml will be enough for 100 students.
   - 100 ml of shampoo without conditioner (3/8 cup). [50 ml of dish detergent can substitute for the shampoo].
   - 15 g sodium chloride (2 teaspoons).
   - 900 ml of water

3. The DNA extraction buffer may be placed at one location and the students can measure out the DNA extraction buffer and add it directly to their bag with the strawberry. Alternatively, the DNA extraction buffer could be dispensed into tubes prior to the start of class and handed to each student.

4. Prior to the start of class, cut the cheesecloth so that it fits over the edge of the funnel. The cheesecloth should be 2 layers thick.

5. The ethanol should be above 90%. It is easiest to have the ethanol in a squirt bottle or dispensed in dropper bottles. They may be kept on ice in one location of the room.

Methods
A. Lysing the cells and nuclei:
1. Obtain one clear plastic bag, 1-2 strawberries, a test tube, test tube rack, funnel, piece of cheesecloth, glass rod or wooden stick, and approximately 10 ml of DNA extraction buffer.

2. Place 1-2 strawberries inside the plastic bag. Use your fist to gently mash the bagged strawberries for 1 minute. This will begin to mechanically break open the cells.

3. Add approximately 10 ml of the DNA extraction buffer to the bagged strawberry. Press the bag so that the air is removed and then seal the bag. Using your fist, gently mash the strawberry in the extraction buffer for 1 minute. This will continue to lyse the cells and to break open the nuclei. The extraction buffer contains shampoo (a source of detergent), salt, and water.

4. Place the cheesecloth onto the funnel to make a filter. Now place the funnel (with the cheesecloth) onto the test tube. Filter the strawberry extract by pouring it onto the cheesecloth and allowing the liquid material to enter into the test tube.

5. Once the test tube is about 1/4 filled stop. Remember to close your zip lock baggie before setting it down and to the side. You may now remove the funnel. The filtration step is necessary to trap the larger cellular materials (broken cell walls, disrupted membrane, organelles) onto the cheesecloth. The DNA extract is filtered into the test tube.

B. Precipitating and spooling the DNA:

1. Obtain an ice-cold ethanol squirt bottle. Gently add the cold ethanol to the cell extract by slowly dripping the ethanol down the side of the tube. The ethanol should layer on top of your filtered cell extract. You should be able to see two distinct layers. The ethanol will be on the top and the cell extract below. Watch at the interface of these two layers. The ethanol will assist in precipitating the DNA. You should begin to see white stringy material (mucous like) forming at the interface of the ethanol and extract. This is DNA.

2. As you are observing the interface, insert a wooden stick or glass rod through the ethanol layer until it reaches the interface of the ethanol and extract. Place the rod just below the interface and twirl or turn the rod. The DNA should attach to the rod and as you twirl it the DNA will wind around the rod. As the DNA winds around the rod it may appear something like white cotton candy.

3. Wash your hands and clean up your laboratory bench putting all materials in the proper place. You may now discard the strawberry extract in the bag into the garbage. Answer the questions on the student data sheet.

Delivery
The student handout provides detailed background information for the student. This activity can be easily modified to increase inquiry-based activities by eliminating the introduction and discussing the concepts with the students. The students could examine cell structure and then be presented with the question of how they would proceed to extract the DNA.

Technology Utilization
Video from the Unseen Life On Earth series describing DNA: Reading the Code of Life
http://www.learner.org/resources/series121.html
ReGenesis http://68.178.137.243/Videofiles/Regenesis/ReGenesis-Final2.mov

Microorganisms
None
Safety Issues
The students should practice standard good laboratory techniques. Goggles should be used.

Assessment and Evaluation of Activity

- Students can complete the Student Data Sheets.
- Students can compose an independent formal lab write up.
- Have students write quiz questions pertaining to the lab and quiz other members of their class. The teacher may also use the student-generated questions to create a test or quiz.
- Journal.
- Presentation (verbal or computer-based).
- Create a concept map that describes the lab and its results.
- Group discussion.
- Use a rubric to assess any of the suggestions above.

Answers to Student Data Sheets

1. Draw an example of a strawberry cell. Where is the DNA located in the cells of a strawberry? Label and mark the location of the DNA inside your cell.

2. What are the cellular structures that serve as a barrier to getting your DNA out of the cell? List them here and label each structure on your drawing above.

   *Cell wall, cell membrane, nuclear membrane, other cellular components that stick to and form complexes with the DNA*

3. Briefly outline the steps required to isolate the DNA from that cell taking the cellular structure into consideration.
   - a) break cell wall, b) lyse cell membrane, c) lyse nuclear membrane, 4) dehydrate DNA

After performing the DNA extraction activity, answer the following:

4. Following you DNA extraction, What did the DNA look like? Describe what you observed after you added the ethanol to the tube:
   *DNA looked like stringy white material at or near the interface. It was very viscous*

5. In this exercise you extracted DNA from cells of the strawberry. What makes the strawberry a good choice to use for the DNA extraction?
   *Plentiful DNA because of its ploidy, ease of extraction because of weakened cell well, little to no nucleases to destroy it, plentiful and inexpensive starting material.*

6. What is the purpose of the detergent (shampoo) in the extraction buffer?
   *Lyse the cell and nuclear membranes*
7. What is the purpose of the salt solution when extracting DNA?

*Dehydrate the DNA*

8. DNA is soluble in water but not soluble in ethanol. Why was this important for the success of the DNA extraction?

*DNA needs to be removed from all the other cellular components. The insoluble materials were left on the bottom of the tube, the soluble DNA was then selectively precipitated from other materials because of its ability to be easily dehydrated with ethanol.*

9. Research scientists who are trying to determine the cause of a genetic disorder would perform a DNA extraction from human tissues. Will the method used to extract DNA from human cells be similar or different from the method to extract DNA from the strawberry? Explain why or why not.

*It will be pretty much the same. However, it should be noted that there are nucleases that can destroy the DNA and they are much more plentiful in some systems. We are also not octoploid so the yield would be much lower per wet weight of tissue.*
Supplementary Materials
This activity has been a modified activity from:

Biology, Exploring Life, Diane Sweeney Labs, Pearson Education

The procedure could be repeated using a different type of tissue. Onion, wheat germ, liver, and thymus are sometimes used. Also, Bio-Rad has a kit in which students extract their own DNA from cheek cells and save it in a small container that hangs on a necklace.

References
DNA Extraction from Strawberry http://www.carlinvilleschools.net/linke/Biology/DNA.htm

Prior to the DNA extraction activity, answer the following:

1. Draw an example of a strawberry cell. Where is the DNA located in the cells of a strawberry? Label and mark the location of the DNA inside your cell.

2. What are the cellular structures that serve as a barrier to getting your DNA out of the cell? List them here and label each structure on your drawing above.

3. Briefly outline the steps required to isolate the DNA from that cell taking the cellular structure into consideration.

After performing the DNA extraction activity, answer the following:

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Introduction
In this laboratory activity you will isolate deoxyribonucleic acid, DNA, from the strawberry. You will learn how cells can be broken open and how the DNA can be separated from the rest of the cellular biological molecules. You will actually see what DNA looks like. This activity brings DNA to life!

Student Background Knowledge
Biotechnology & Applications:
Biotechnology is the use and manipulation of living organisms to provide a practical outcome or product that should benefit mankind. Biotechnology is a broad term that may include different laboratory procedures and applications. Modern biotechnology often involves the manipulation of the deoxyribonucleic acid, DNA from living organisms. These applications that involved the manipulations of DNA is referred to as recombinant DNA technology or genetic engineering. Recombinant DNA technology generally involves the isolation of the DNA or genes, the alteration of genes, and the ability to move genes from one organism to another.

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The chromosomal DNA is the blueprint for an organism determining what that organism will look like. DNA contains all the genetic information that forms the basis of inheritance and directs the making of proteins. The strawberry is a eukaryotic plant cell containing the chromosomal DNA inside the nucleus. In order for one to be able to extract and visualize the DNA, one must have a large quantity of DNA inside the test tube. This means that the DNA must be extracted from a large number of cells. A single strawberry contains many cells providing a good cellular sample for DNA. The strawberry is octoploid meaning that it contains eight copies of each type of chromosome and this provides a large amount of DNA that can be isolated from one or two strawberries.

What are the basic steps for extracting DNA from eukaryotic cells such as a plant cell? The entire process can be divided into four basic steps. There are special procedures and reagents that are used to accomplish each of these steps. The four steps include: disrupting the cell wall, lysing the cytoplasmic membrane (also called the plasma membrane), lysing the nuclear envelope, and separating out the DNA from the rest of the cellular material.

First the cell wall and cytoplasmic membrane surrounding the plant cell must be disrupted or broken open. The process of “breaking open” the cell is referred to as “lysing the cell”. Once the cytoplasmic membrane is lysed the nucleus is released. Since the nuclear envelope surrounds the DNA, this membrane must also be broken open to completely release the DNA. Lysing the plasma membrane and the nuclear envelope can be accomplished together since both are membranes with a similar composition of lipids and proteins. Detergents are useful reagents that assist with disrupting the membranes. The molecules that make up the detergent interact with the lipids and proteins and act to pull them away from the membrane. Therefore one can easily disrupt membrane structure to lyse the cytoplasmic membrane and nuclear envelope by manually squashing the strawberry and adding a detergent.

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Following the lysing of the plasma and nuclear membranes, a cell lysate (extract) has been generated which contains the released DNA along with all the other cellular components. The next step is to separate out the DNA from all of the other molecules of the cell. The DNA is precipitated and thereby separated by using ethanol and salt. Recall that the DNA molecule is double stranded and each strand is composed of a phosphate-sugar backbone (the sides of the ladder) with the nitrogen containing bases that hydrogen bond with the bases from the other strand (the rungs). The outer phosphate groups on the phosphate backbone provide negative charges that can hydrogen bond with water molecules. The ability of DNA to hydrogen bond to water molecules allows DNA to be soluble in water. Ethanol carries a hydroxyl group that is capable of forming hydrogen bonds with water. When ethanol is added to the cell lysate, the ethanol forms hydrogen bonds with water molecules and pulls the water away from the DNA. The DNA is insoluble in ethanol. The negative charges in the DNA will interact with the charges from the salt. Since the DNA can no longer interact with water molecules, it aggregates with other DNA molecules and salt thereby precipitating or separating out from the rest of the cellular components. Following the addition of the ethanol and salt, the DNA precipitates and appears as a stringy white substance.
Vocabulary
Review the meaning of the following words: biotechnology, recombinant DNA technology, genetic engineering, deoxyribonucleic Acid (DNA), plant cells, extraction, precipitate, hydrogen bonding.

Safety Considerations
Use standard safe laboratory practices. For the safety of your eyes wear goggles.

Materials Checklist
- 1-2 strawberries in a plastic bag
- 1 test tube
- 1 test tube rack
- funnel
- small piece of cheesecloth
- 10 ml of DNA extraction buffer
- Ethanol in a squirt bottle (keep on ice)
- Glass rod or wooden stick

Procedure

A. Lysing the cells and nuclei:

4. Obtain one clear plastic bag, 1-2 strawberries, a test tube, test tube rack, funnel, piece of cheesecloth, glass rod or wooden stick, and approximately 10 ml of DNA extraction buffer.

5. Place 1-2 strawberries inside the plastic bag. Use your fist to gently mash the bagged strawberries for 1 minute. This will begin to mechanically break open the cells.

6. Add approximately 10 ml of the DNA extraction buffer to the bagged strawberry. Press the bag so that the air is removed and then seal the bag. Using your fist, gently mash the strawberry in the extraction buffer for 1 minute. This will continue to lyse the cells and to break open the nuclei. The extraction buffer contains shampoo (a source of detergent), salt, and water.

7. Place the cheesecloth onto the funnel to make a filter. Now place the funnel (with the cheesecloth) onto the test tube. Filter the strawberry extract by pouring it onto the cheesecloth and allowing the liquid material to enter into the test tube.

8. Once the test tube is about 1/4 filled stop. Remember to close your zip lock baggie before setting it down and to the side. You may now remove the funnel. The filtration step is necessary to trap the larger cellular materials (broken cell walls, disrupted membrane, organelles) onto the cheesecloth. The DNA extract is filtered into the test tube.

C. Precipitating and spooling the DNA:

1. Obtain a squirt bottle containing ice-cold ethanol. Gently add the cold ethanol to the cell extract by slowly dripping the ethanol down the side of the tube. The ethanol should layer on top of your filtered cell extract. You should be able to see two distinct layers. The ethanol will be on the top and the cell extract below. Watch at the interface of these two layers. The ethanol will assist in precipitating the DNA. You should begin to see white stringy material (mucous like) forming at the interface of the ethanol and extract. This is DNA.

2. As you are observing the interface, insert a wooden stick or glass rod through the ethanol layer until it reaches the interface of the ethanol and extract. Place the rod just below the interface and swirl or turn the rod. The DNA should attach to the rod and as you
twirl it the DNA will wind around the rod. As the DNA winds around the rod it may appear something like white cotton candy.

3. Wash your hands and clean up your laboratory bench putting all materials in the proper place. You may now discard the strawberry extract in the bag into the garbage. Answer the questions on the student data sheet.
**Extracting DNA from Strawberries**

**Student Data Sheet**

Name: ___________________________ Date: ___________________________

*Prior to the DNA extraction activity, answer the following:*

1. Draw an example of a strawberry cell. Where is the DNA located in the cells of a strawberry? Label and mark the location of the DNA inside your cell.

2. What are the cellular structures that serve as a barrier to getting your DNA out of the cell? List them here and label each structure on your drawing above.

3. Briefly outline the steps required to isolate the DNA from that cell taking the cellular structure into consideration.

*After performing the DNA extraction activity, answer the following:*

4. Following you DNA extraction, What did the DNA look like? Describe what you observed after you added the ethanol to the tube:

5. In this exercise you extracted DNA from cells of the strawberry. What makes the strawberry a good choice to use for the DNA extraction?

6. What is the purpose of the detergent (shampoo) in the extraction buffer?

7. What is the purpose of the salt solution when extracting DNA?
8. DNA is soluble in water but not soluble in ethanol. Why was this important for the success of the DNA extraction?

9. Research scientists who are trying to determine the cause of a genetic disorder would perform a DNA extraction from human tissues. Will the method used to extract DNA from human cells be similar or different from the method to extract DNA from the strawberry? Explain why or why not.