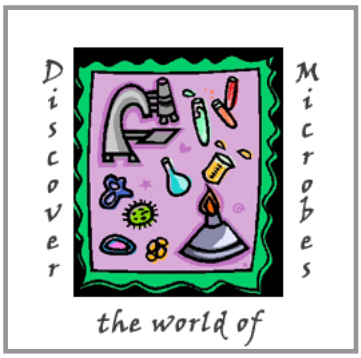
Microbial Discovery Activity

Extracting DNA from a Banana



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Intended Audience

K-4	X
5-8	X
9-12	

(*This lesson is most appropriate for students in grades 5-8. However, it may also be slightly adapted to allow younger students to complete the activity.)

Activity Specifications

Classroom setting	X
Requires special equipment	X
Uses hands-on manipulatives	Liquid handling and solid manipulation
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

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Introduction

Description
Students use a high salt/detergent mixture to solubilize a piece of a banana, then add cold ethanol to precipitate a froth of white DNA from solution.

Abstract
The soft flesh of a banana provides a ready source of DNA. Using a few simple purification steps in a classroom setting, students can yield loads of crudely prepared DNA. To begin, the banana is mashed in a detergent/salt solution to lyse the cellular and nuclear membranes. Cellular lysate is strained, then the solubilized DNA is cleansed with a meat tenderizer (which contains an enzyme that breaks apart proteins). Lastly, ethanol is added to generate soft, white, globs of DNA and perhaps – with careful technique – slender threads that may be wound onto a glass rod.

Core Themes Addressed

Microbial Cell Biology	
Microbial Genetics	
Microorganisms and Humans	
Microorganisms and the Environment	
Microbial Evolution and Diversity	
Other -Common properties of life; Cellular components	X

Keywords
Lysis, Solubilize, Precipitate, Cell component, DNA

Learning Objectives

At completion of this activity, learner will:

1. have successfully extracted DNA from a banana, given the materials provided.
2. observe, handle, and describe a crude preparation of life's hereditary material.
3. have the opportunity to use prior and newly acquired knowledge to draw conclusions regarding the structure and function of DNA.
4. have separated cellular components according to the standard scientific approach of exploiting chemical differences between the molecules of the cellular milieu.

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 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. Students explore DNA as being the molecular basis of heredity

Teacher Handout

Extract DNA from a Banana

Student Prior Knowledge

Students may have prior knowledge of DNA's structure and function.

For students who have prior knowledge of the nature of DNA, the addition of cold ethanol at the end of the protocol provides an impressive moment when the white goo of DNA appears so suddenly and in such quantity. Contrasting starkly with the clear liquid extract from the cells, students may be pleased to be able to see for themselves this substance that they know to control life's processes.

For students who have no prior knowledge of the nature of DNA prior to beginning the activity, their own preparation of DNA may serve as an intriguing lead-in to a more conceptual discussion about the structure and function of the genetic material.

Class Time

This lesson will require a minimum of one 45 minute class period.

1. The initial step to mash the banana in the solubilizing fluid requires only a few minutes. Small chunks of banana may remain, since even if many cells fail to be effectively solubilized, the amount of DNA that will be released is vast.
2. Filtration of the cell extract through the cheesecloth is the slowest step. Plan to allow 10-15 for the banana mash to filter. This should produce approximately 10 to 15 ml of filtrate, which is sufficient for the 6 ml of ethanol in the last step.
3. The white masses of DNA will begin to precipitate immediately. If the students' DNA will be able to spool onto the glass stirring rod, it will do this almost immediately after addition of the ethanol. If the DNA has become too fragmented during the protocol(for example, due to shearing during mixing steps), then no amount of spinning the glass rod will help to wind the DNA. It is either intact--or not--by the time the ethanol is added. Still, even if the fragments are not long enough to wind onto the rod, they will constitute an impressive amount of soft blobs in the students' mixtures.

Teacher Preparation Time

This lesson will require approximately 20-25 minutes of preparation time.

1. Two solutions must be prepared in advance.
   Solubilizing solution consists of 10% NaCl (wt/vol) plus 10% detergent (vol/vol) in water Meat Tenderizer solution consists of an aqueous suspension of 5% (wt/vol) tenderizer
2. Ethanol (95%) should be placed into a freezer (or refrigerator or icebath) in advance of lab to chill it down. Cold ethanol precipitates the DNA better than warm ethanol. Teachers may want to aliquot the DNA into 6-ml portions for the students or have the students work quickly to measure out their own 6-ml portions from a larger vessel of cold ethanol.
3. Cheesecloth squares could be cut in advance. For example, for each banana piece, students might use two layers of cheesecloth, approximately 15 cm on each side. The double thickness of cheesecloth is sufficient to retain the solids from the banana mash.

Materials and Equipment

For each student or group of students who will be extracting DNA, the following will be used:

1. Fresh banana piece (about 2 cm cube)
2. Mortar & pestle (OR spoon)
3. 2 beakers (150-250 ml sizes) If no beakers are available, clear plastic drinking cups can be substituted
4. Graduated cylinders – 10 ml & 100 ml
5. Cheesecloth squares
6. Solubilizing (detergent/salt) solution
   [To prepare 200 ml of this solution (sufficient for 10 pieces of banana), dissolve 20 g of non-iodized salt into 180 ml of distilled water, then gently mix in 20 ml of liquid detergent (such as a liquid dishwashing detergent), avoiding bubble formation]
7. Meat tenderizer solution
   [To prepare 100 ml of this solution (sufficient for 20 pieces of banana), mix 5 g meat tenderizer into 95 ml distilled water. The liquid will be cloudy. McCormick Brand unseasoned tenderizer (containing salt, dextrose, the enzymatic component (bromelain), and calcium silicate) is one brand that has been shown to work in this protocol. Five grams is equivalent to just a little over one teaspoon.]
8. Funnel OR rubber band for holding the cheesecloth
9. Cold ethanol (95%) [6 ml per banana piece used]
10. Glass stirring rod
11. Large test tube and test tube rack (or other holder). These items may be omitted if the beaker is clear.

Methods

1. Obtain a fresh piece of peeled banana and mash it with the mortar and pestle.
2. Combine the mashed banana with 20 ml of detergent/salt solution in a beaker and stir.
3. Strain the mixture into the second beaker with a piece of cheesecloth.
4. Add 5 ml of meat tenderizer solution to the banana solution and stir.
5. Pour the banana solution into the test tube.
6. Pour 6 ml of cold ethanol on top of the banana solution in the test tube.
7. Let the test tube sit until the bubbling stops. [This is a good time to wash the supplies.]
8. The DNA is now floating at the top of the alcohol layer. Carefully swirl a glass rod in the floating DNA. You should be able to see small "threads" of DNA.
9. Draw and label the test tube and its contents.

Notes on the steps described above:

1. The banana may be pleasantly ripe or over-ripe. If no mortar and pestle is available, the banana can be mashed in the beaker with a spoon.
2. Steps #1 and #2 can be combined by pouring the 20-ml of solubilizing solution directly onto the banana and mashing it into a thick liquid. Small chunks of banana are okay as long as most of the mixture is a smooth, liquidy consistency.
3. If no funnels are available to hold the cheesecloth, it can be placed over the second beaker and fastened with a rubber band. Then the banana mash can be poured on top and left it for a few minutes, until a sufficient volume collects in the clean beaker. The strained liquid will look nearly clear with a faint tan color. The minutes while the students wait for the liquid to strain might be a spent discussing how the detergent and salt mixture has destabilized the membranes, allowing the release of each cell's contents, including DNA from the nucleus and proteins from the cytoplasm (note that some of the proteins are used by cells to recycle DNA, so the meat tenderizer in the next step will help to inactivate enzymes that might otherwise attack the released DNA).
4. Very gently mix the meat tenderizer solution into the filtrate. DNA that becomes sheared will not spool onto the glass rod at the end of the procedure.

5. Although the protocol calls for pouring the mixture into a test tube at this point, this step can be skipped if the student is using a transparent beaker and can clearly see the liquids inside. It is sometimes possible to spool out the DNA, however due to shearing by the protocol it is not always possible. One can typically notice a large white precipitate at the interface.
6. If omitting the test tube, students can place their glass rod into the filtrate (in a beaker) and add the cold ethanol by allowing it to trickle down the rod. After all the ethanol has been added, twirl the rod to help pull the DNA out of the lower aqueous phase and into the ethanol layer that is on top. DNA will spool onto the glass rod if it has not become too fragmented in earlier steps. DNA that has precipitated can be seen floating in the liquid. Looking closely, fine threads of DNA can be noted wound onto the glass stirring rod.

Delivery

"Extract DNA from a Banana" is a laboratory activity that can be conducted by students alone, in pairs or in small groups, depending on availability of laboratory glassware

Technology Utilization

Neither computers nor calculators are required for this laboratory activity.

Microorganisms

This lesson focuses on the extraction of DNA and does not contain any relevant microorganisms.

Safety Issues

Students will be working with common household chemicals. It is important to emphasize the importance of laboratory safety. Also, students will be working with laboratory glassware. Be sure to inform students of glass disposal procedures.

Assessment and Evaluation of Activity

Students may be informally assessed for their ability to follow directions and complete the laboratory activity. A formal assessment may consist of several different options. The instructor may require students to record data, write a description, or answer a question on a test. Also, the instructor may simply base their formal assessment on the students' answers to the follow-up questions.

Supplementary Materials

Possible Modifications: For students in lower grades, the DNA may be previously extracted, allowing the younger students the opportunity to observe the final product, yet not complete the steps to extraction.

References

None provided

Answers to Student Data Sheets

1. What are the parts of DNA?

There are many ways to answer this question, depending upon a student's understanding of the word, "parts". In terms of DNA chemistry, students might think of four chemical parts, which are the four kinds of nucleotides that are used to make DNA. These nucleotides are usually called A (for adenine), C (for cytosine), G (for guanine), and T (for thymine). If students are thinking of DNA as a double helix, then they might imagine the shape of a very long twisting ladder. In this way of thinking, there might be two parts (the rungs of the ladder and the long supports for the rungs). This visualization helps to emphasize the A-T pairings and the C-G pairings that form each rung, as well as the lengthwise split down the middle of each rung which can occur when DNA replicates or is transcribed into mRNA. Or, students might think of the functional aspect of DNA, in which one part of DNA represents the thousands of genes that code for something in the cell, with other parts of DNA, those that don't code for useful information, interspersed between the genes. No matter how students imagine the DNA's parts, the DNA is long: millions of nucleotides long, millions of ladder rungs, or thousands of genes, each with of hundreds of thousands of nucleotides.

2. Would DNA from a different source look different? Why or why not?

DNA is DNA, so if obtained from something other than a banana it would not look different. DNA differs from one organism to the next because of the different order of the nucleotides. This different order defines different genetic information, but it cannot change the overall structure of the DNA on a large scale as seen by the masses of white precipitate produced in this exercise.

3. Why does DNA appear as a viscous material?

DNA is very long, and able to stick to itself via AT and GC pairings. The many pieces of DNA, all joined together at different places by regions of base pairing, make the individual "strands" mesh together to resemble a goo.

4. Why would a solution become more viscous after lysis of cells?

Once the membranes have been solubilized, the DNA is no longer contained within a cell. Then, it can find many other molecules of DNA that have been released from other cells. These stick to each other to make large globs composed of DNA released from millions of cells. It is also very long and compact when inside a cell. When released from those confines it can spread out and interact with other molecules.

5. How long is the DNA in an individual cell and how does the length of DNA compare to the size of a cell?

DNA in human cells is generally said to be about one meter in length. Amazingly, the length of the cell that normally contains that DNA is about 0.01 mm in diameter. To fit into its cell, each DNA molecule has to be twisted and compacted into less than one hundred-thousandth its length (1000 mm compacted into 0.01 mm). NOTE: teachers may wish to check out the classic image of DNA release from a bacterium to illustrate this concept. Most microbiology textbooks include such a micrograph. Or, check out an on-line image at http://www.bact.wisc.edu/themicrobialworld/bactDNA2.jpg

6. What are the roles of each of the components in the lysis solution?
Salt and detergent each work to dissolve the bilipid layer of membranes and release the cellular contents, including DNA. Meat Tenderizer contains enzymes that attack proteins. This helps to protect the DNA from enzymes that might cut the DNA and also helps to get rid of other proteins (such as histones) which might be compacting the DNA. Ethanol dehydrates the DNA by removing the water. This dehydrated molecule then forms a precipitate, which means that it separates from other materials in the liquid.

7. Why use banana as a source of DNA?
Every cell has DNA. Any fruit has lots of cells, therefore lots of DNA. Bananas are soft and dense, without a lot of stringy or gritty material which might be present in some fruits (imagine a pear, for instance). Their softness makes it easy to release their DNA without a lot of work.

8. What are some other materials that would be a good source of readily isolated DNA? Can you propose any biological materials that would not be such good sources for DNA??
Any material that contains lots of cells would be a possible source of DNA. Bakers' yeast and bacterial cultures contain lots of cells. Seeds and grains might also be good, since these probably have lots of cells that are ready to germinate into next year's plant. However they are hard to grind up so not used very often. Animal cells, too, contain DNA: one easy source of animal cells is the cheek cells from students' rinsing their mouths! On the other hand, nuts would not be as great sources of DNA since they contain a lot of oils and proteins as storage materials for the growing plant. A hen's egg would be a terrible source of DNA since the whole thing contains lots of storage materials to nourish a growing chick but only a single cell (thus, very little DNA). Sperm is an excellent source of DNA since it has very few other cellular components. However in practical terms some sources are easier to acquire than others.

9. Name some parts from the banana cells that became trapped in the cheesecloth and discarded:
Cell walls and portions of membranes would be stuck in the cheesecloth. Also, whole cells that failed to be lysed by the solubilizing solution would also be stuck in above the cheesecloth. DNA, proteins, mRNA and any small components from cytoplasm would have passed easily through the filter. Most of the filtrate is the solubilizing solution, into which the soluble components from banana cell cytoplasm flowed.

10. The smallest thread that can seen by the human eye is about 0.2 mm thick. Yet the diameter of the DNA double helix is much, much thinner: only about 2 nm. If you were able to see threads of DNA on your glass rod, it means that you were looking at many DNA molecules lined up together to increase the thickness of the group.

Can you estimate how many strands of DNA double helix would have to bundle together to add up to a diameter large enough to see on the glass rod? Hint: remember that it takes 1000 nm to equal one micrometer, and it takes 1000 micrometers to equal one mm.
About 100,000 DNA helices bundled together would give a thickness of 0.2 mm.
To solve the equation for "?":

? threads =(1 thread / 2 nm) x (1000 nm / 1 micrometer) x (1000 micrometers / 1 mm) x 0.2 mm

Student Handout

Taking the Mystery out of DNA: Extracting DNA from Strawberries

Introduction

DNA, or deoxyribonucleic acid, is found in the cells of all living things. It is the master code or blueprint for the organism. During cell division, this code is copied and passed to new cells. DNA also controls all cellular activities through its role in protein synthesis.

Today, we'll be extracting DNA from a banana. To do this, we must release the DNA from the cell by breaking apart, or lysing, the cellular and nuclear membranes. This is performed by mashing the banana and adding a detergent/salt solution. The DNA is then cleansed with the meat tenderizer. This contains an enzyme that breaks apart proteins. Lastly, we add alcohol, allowing the DNA to uncoil and precipitate out.

Student Background Knowledge

To prepare for this lab activity, students should find a diagram or image of a plant cell in their book or on-line references. Make a sketch of this cell in the space below. Then label your sketch to show the location of the DNA and the location of the membranes that will be lysed during this procedure. Also label the location of the cell wall, a structure that is strong but leaky (this wall will not really be affected by the steps in our procedure).

Vocabulary

DNA: Deoxyribonucleic acid, the molecule that cells contain, carries genetic information allowing for reproduction and cell division.

Lysing: In this case, the act of breaking open the cell membranes to expose the contents.

Membrane: "A living layer that cells produce to organize and contain life's processes. Membranes in a banana cell include the cell membrane to separate each cell from its environment and the nuclear membrane, to contain the DNA within each cell"

Precipitate: Formation of a solid during a chemical reaction.

Safety Considerations

Be careful when working with chemicals. As always, do not place any laboratory materials in your mouth. Although some materials may be safe to eat under other circumstances, never consume food during a lab without teacher permission. Also, be extremely careful when handling laboratory glassware. Tell your instructor if something breaks. Do not clean it up by yourself.

Materials Checklist

	Fresh banana piece (peeled, about 2 cm cube)
	Mortar & pestle
	2 beakers
	Graduated cylinders – 10 ml & 100 ml
	Cheesecloth
	Funnel or rubber band
	Glass stirring rod
	Solubilizing solution [made of 10%detergent and10% non-iodized salt in water. You will need 20 ml
	Meat tenderizer solution 5% meat tenderizer in distilled water. You will need 5 ml
	Cold Ethanol. You will need 6 ml
	Screw-capped test tube and rack to hold the test tube (optional)

Procedure

1. Obtain a piece of banana that is peeled and measured to the right size..Mash it with the mortar and pestle.
2. Combine the mashed banana with 20 ml of detergent/salt solution in a beaker and stir.
3. Strain the mixture into the second beaker through a piece of cheesecloth. After a few minutes (or after approximately 10-15 ml of liquid has collected), discard the cheesecloth together with the banana mush inside the cloth.
4. Record the appearance of the liquid that you have collected.:

_____

5. Add 5 ml of meat tenderizer solution to the banana solution and stir gently.

6. Place the glass stirring rod into the liquid. Pour 6 ml of cold ethanol into the beaker of banana solution by allowing the ethanol to run down the rod.
7. Describe your observations after the ethanol has been added to the solution.:

_____

8. Carefully swirl the glass rod in the floating DNA. You may be able to see small "threads" of DNA wind onto the rod.
9. Record your results in the space below
10. After your observations have been recorded, wash the glassware.

Results

Draw the liquids and solids as these appear in your beaker. Describe the appearance of the DNA (color, texture, quantity, how well it sticks to the rod, etc).. Label the DNA in your drawing

Extract DNA from a Banana
Student Data Sheet

Name: _____ Date:_____

1. What are the parts of DNA?

2. Would DNA from a different source look different? Why or why not?

3. Why does DNA appear as a viscous material?

4. Why would a solution become more viscous after lysis of cells?

5. How long is the DNA in an individual cell and how does the length of DNA compare to the size of a cell?

6. What are the roles of each of the components in the lysis solution?

7. Why use banana as a source of DNA?

8. What are some other materials that would be a good source of readily isolated DNA? Can you propose any biological materials that would NOT be such good sources for DNA?

9. Name some parts from the banana cells that became trapped in the cheesecloth and discarded:

   Name some parts of the banana cell that passed through the cheesecloth and were collected in the solution:

10. The smallest thread that can seen by the human eye is about 0.02 mm thick. Yet the diameter of the DNA double helix is much, much thinner: only about 2 nm. Can you estimate how many strands of DNA double helix would have to bundle together to add up to a diameter large enough to see? Hint: remember that it takes 1000 nm to equal one micrometer, and it takes 1000 micrometers to equal one mm.