



Dolan
DNA Learning Center
www.dnalc.org

DNA Extraction



Background

DNA is found within a nuclear membrane in *eukaryotic* cells. Almost all living things are composed of eukaryotic cells. Bacteria and cyanobacteria lack a nuclear membrane. In these *prokaryotic* cells, DNA is found floating concentrated within the cytoplasm. The term prokaryotic is derived from “pro” meaning before and “karyote” meaning kernel. The kernel refers to the nucleus as it appears under a light microscope.

In this experiment, students extract DNA from a strain of *E.coli* (*Escherichia coli*) bacteria called mm294. This is a harmless laboratory strain. *E.coli* bacteria live in the human colon with hundreds of other bacterial strains. In the human gut, bacteria digest material that would have been eliminated as fecal matter and are a source of Vitamin K. This example of a mutualistic relationship between two organisms demonstrates that bacteria are not all harmful. In fact, most bacteria are harmless to humans. Some relationships are parasitic. In cases of food poisoning, *Salmonella* bacteria or harmful strains of *E. coli* (strain 0157:H7) bacteria are pathogenic. *Salmonella* bacteria may be found in poultry or eggs and harmful *E.coli* in red meats. Proper storage and cooking of these foods can greatly reduce the risk of developing food poisoning.

To extract DNA from bacterial cells, the outer membrane must be penetrated. Cell membranes are essentially phospholipid bilayers. The “heads” and “tails” of the phospholipids and their affinity for water affect the structure of the lipid bilayer. Phospholipids are visually represented as molecules with sphere shaped “heads” and double lined “tails”. The heads are hydrophilic which means they “like” water whereas the tails are hydrophobic meaning they “dislike” water. Because of their similarities, the phospholipid tails face inward and the heads face outward. This creates a waterproof lipid bilayer around the circumference of the cell. It is semi-permeable, which means that it allows certain substances to flow freely between the inside and outside of the cell, while other substances cannot. Proteins and carbohydrates are also important parts of the cell membrane. Proteins are embedded throughout the lipid layers like mosaic tiles and allow for the movement of large molecules into and out of the cell. Carbohydrate side chains, called cell receptors, that allow cells to communicate with each other can be found on the outer surface of the cell.

Lipids are the main component and are the target of destruction in this activity. Lipids are classes of fats, waxes and oils. The most common tools for disrupting lipid membranes are detergent and heat. Detergent molecules are structurally similar to lipid molecules. This similarity creates an attraction between them. When cells are combined with detergent, it literally sticks to the lipid membrane surrounding the cell and destroys it by pulling the lipids away from each other. When the membrane is destroyed, the contents of the cell are released. In the presence of heat, the process speeds up.

Once DNA is extracted from bacteria cells, it becomes visible without a microscope. This is only possible when a large number of cells are used, thus yielding a large number of DNA strands.



Description of Activity

In this one-hour activity, children in grades 5 through 8 will learn how to extract DNA from prokaryotic cells and have the opportunity to see DNA without a microscope. Students will review the structure and function of DNA and discuss why scientists and other professionals might want to extract DNA from various sources. This lesson is also an opportunity to compare prokaryotic and eukaryotic cells and review basic cell structures.

Learning Outcomes

Students will:

- learn a basic procedure for extracting DNA from cells.
- explain the differences between eukaryotic and prokaryotic cells.
- describe the significance of understanding cell composition in a DNA extraction.
- use prior knowledge of cell structures to determine the most efficient method for extraction from prokaryotic cells.
- see real DNA without a microscope.

Assumptions of Prior Knowledge

Students should have an understanding of the structure and function of the DNA molecule and a basic understanding of the structure and function of a cell.

Misconceptions

Many students assume that all bacterial strains are infectious. Students might also assume that they will be able to see the double helix without the help of a microscope.

Lesson

Materials and Equipment

- 5 ml bacterial culture in a plastic test tube with a screw cap-1 per student pair
- Transfer pipettes-1 per student pair
- Water bath- temperature between 65-75°C -1 per class
- Small racks or large cups to hold materials vertically
- Clear plastic inoculating loops-1 per student pair
- Permanent markers-1 per student pair

Reagents

- 3 ml Palmolive dishwashing detergent – 1 per student pair
- 3 ml ethanol- 1 per student pair

Recipes

Luria-Bertani (LB) Broth (1L)

- 10g Tryptone
- 5g-yeast extract
- 10g NaCl
- 875 μ l 4N NaOH (4g NaOH /100ml of dH₂O)
- Mix ingredients into 1L of dH₂O.
- Pour into glass bottles (100ml each).
- Autoclave for 25 minutes.

Basic MM294 (*E.coli*) overnight culture

- Aliquot 2ml of sterile LB broth into a 15ml “snap-cap” tube.
- Use a sterile, medium-size pipette tip to pluck a colony from a fresh plate of desired bacteria.
- Eject tip directly into LB aliquot.
- Incubate tube overnight at 37°C in a SHAKING incubator. Make sure tube caps are loose so that cells can aerate.

MM294 (*E.coli*) cultures for DNA extraction

- Inoculate 100ml of sterile LB broth with a fresh overnight culture of mm294.
- Make 20-5ml aliquots in 15ml coming tubes.
- Incubate tubes overnight at 37°C in a SHAKING incubator. Make sure tube caps are loose so that cells can aerate.

Purchasing Information

- *Escherichia coli*, strain mm294-Carolina Biological Supply Company
- Snap-cap tubes (Falcon 5059)-VWR International
- Plastic rods (plastic inoculating loops)- VWR International
- Transfer pipettes- VWR International
- Miscellaneous chemicals – Fisher Scientific

Before Class

- Prepare bacterial cultures as described above.
- Photocopy worksheets for each set of students.



- Prepare water bath. Temperature should range from 65-75°C.
- Set up stations with appropriate material for each pair of students. This should include a rack, a permanent marker, soap, ethanol, bacterial culture, a dropper and a plastic rod.

During Class

- Discuss some basic characteristics of bacteria (unicellular, prokaryotic, rapid division).
- Review the cellular structures within animal and bacteria cells, and discuss the similarities and differences between prokaryotic and eukaryotic cells.
- Introduce the bacteria being used for the experiment (*E.coli*). Discuss the numerous strains that exist, such as pathogenic strains, strains that live in your body and the harmless strains that are used for research (the latter be used for this experiment).
- Ask students how they might extract DNA from prokaryotic cells. They should realize after observing diagrams and discussing cellular structures that the outer membrane is the only barrier between a bacterial cell's DNA and the outside environment.
- Point out that breaking through a cell membrane can be compared to breaking through a wall. If a builder needed to knock down a wall, how would he or she determine which tools might work best? What specifically would the builder need to know about the wall itself?
- As a class, examine the components of the cell membrane. Make sure that the students understand that lipids are molecules of fat. If a membrane is made primarily of fat molecules, what kind of tool could be used to break it down, or dissolve it?
- Discuss the fact that household soaps/detergents are used all the time to dissolve fat from greasy dishes, skin and laundry. In this experiment, dishwashing detergent dissolves the prokaryotic cell membrane in the same way, thus releasing DNA from the cell.
- Read steps 1-4 aloud, demonstrate, and give students the opportunity to begin their experiments in small groups.
- During the 15-minute incubation, discuss what is happening inside all of the tubes, and what will need to be done next. Once the DNA is released from the cells, it needs to be precipitated from the soapy solution in which it lies.
- Read step 5, and draw a diagram of precipitation with ethanol. Make sure students understand that ethanol is a type of alcohol, similar to rubbing alcohol. Because of its chemical properties, DNA is not soluble in ethanol, and it can therefore be used to

separate the DNA from the soapy solution in the tube.

- Demonstrate how to use the transfer pipette to add ethanol. Slowly pour ethanol down the inside of the experiment tube and emphasize the importance of holding the tube on an angle.
- Show students how to spool the DNA from the ethanol layer using a plastic rod without breaking through the soap layer. It is essential that the two layers remain separated.
- If students would like to keep their DNA samples, they can be stored in small tubes of ethanol indefinitely.

Analysis and Discussion

- Review the characteristics of bacteria and the benefit of using a prokaryotic organism for a DNA extraction.
- Ask students how it was possible to see real DNA without the help of a microscope, and how James Watson and Francis Crick were able to determine the double helical shape of the DNA molecule.
- Discuss the fact that the DNA extraction is an experiment that is not only used by scientists, but also by detectives (fingerprinting) and doctors (disease diagnosis).

Further Explorations

Use a different protocol to extract DNA from eukaryotic cells, such as fruit. Compare and contrast the results.

Students can research a variety of plants and/or animal species to determine the chromosome number in each. If an organism has more chromosomes, does that mean that it is more complex? What portion of one organism's genes are the same as another's?

Have students research the history of DNA fingerprinting and assign a different case to each group of three to four students. Allow the students to share what they learn with their classmates in an open group discussion.

Explore the *Recovering the Romanovs* activity on DNAI, at: www.dnai.org > Applications > Recovering the Romanovs. Students can learn how DNA extracted from skeletal remains can be used to identify individuals from the Romanov family of Russia, and solve the mystery of the missing Princess Anastasia themselves by analyzing mitochondrial DNA sequences online!



Resources

Web Sites:

<http://www.bacteriamuseum.org/main1.shtml>

The Virtual Museum of Bacteria

<http://www.dnafb.org>

The Dolan DNA Learning Center's Internet site.

Use this site to explore various genetics concepts from inheritance to genetic engineering.

<http://www.dnai.org>

The Dolan DNA Learning Center's Internet site.

Use this site to learn about the past, present and future of DNA science.

<http://www.ygyh.org>

The Dolan DNA Learning Center's Internet site.

Use this site to learn about the connection between genes and health.

Books:

Enjoy Your Cells, Dr. Fran Balkwill, Carolrhoda Books, Inc., MN 2002

Cells are Us, Dr. Fran Balkwill, Carolrhoda Books, Inc., MN 1990

Have a Nice DNA, Dr. Fran Balkwill, Carolrhoda Books, Inc., MN 2002

The Way Life Works, Mahlon Hoagland & Bert Dodson, Times Books, NY 1995

Correlations

New York State

NYS Standard 4: Science

The Living Environment

- Living things are both similar to and different from each other and nonliving things.
- Organisms inherit genetic information in a variety of ways that result in continuity of structure and function between parents and offspring.
- The continuity of life is sustained through reproduction and development.
- Human decisions and activities have had a profound impact on the physical and living environment.

National

Content Standard C: Life Sciences

Structure and functions in living systems

- Living systems at all levels of organization demonstrate the complementary nature of structure and function. Important levels of organization for structure and functions include cells, organs, tissues, organ systems, whole organisms and ecosystems.
- All organisms are composed of cells—the fundamental unity of life. Most organisms are single cells; other organisms, including humans, are multi-cellular.
- Cells carry on the many functions needed to sustain life. They grow and divide, thereby producing more cells. This requires that they take in nutrients, which they use to provide energy for work that cells do and to make the materials that a cell or an organism needs.

AAAS Benchmarks

Standard C: Cells

- Some living things consist of a single cell. Like familiar organisms, they need food, water and air; a way to dispose of waste; and an environment they can live in.
- Microscopes make it possible to see that living things are made mostly of cells. Some organisms are made of a collection of similar cells that benefit from cooperating. Some organisms' cells vary greatly in appearance and perform very different roles in the organism.



Description of Activity

In this one-hour activity, children in grades 5 through 8 will learn how to extract DNA from eukaryotic plant cells, and have the opportunity to see DNA without a microscope. Students will review the structure and function of DNA and discuss why scientists and other professionals might want to extract DNA from various sources. This lesson is also an opportunity to review basic cell structures.

Learning Outcomes

Students will:

- learn a basic procedure for extracting DNA from cells.
- use prior knowledge of cell structures to determine the most efficient method for extraction from eukaryotic cells.
- describe the significance of understanding cell composition in a DNA extraction.
- see real DNA without a microscope, and explain how this is possible.

Assumptions of Prior Knowledge

Students should have an understanding of the structure and function of the DNA molecule and a basic understanding of the structure and function of a cell.

Misconceptions

Students might assume that they will be able to see the double helix without the help of a microscope.

Lesson

Materials and Equipment

- 5 ml pureed fruit (baby food works well)
- Transfer pipettes-1 per student pair
- Water bath-1 per class
- Small racks or large cups to hold materials
- Clear plastic inoculating loops-1 per student pair
- Permanent markers-1 per student pair
- Paper coffee filters – 1 per student pair
- Disposable plastic cups – 1 per student pair
- Clean snap cap tubes – 1 per student pair

Reagents

- 3 ml soap buffer solution– 1 per student pair
- 3 ml ice cold ethanol- 1 per student pair

Recipes

Soap Buffer Solution

- 7.5g NaCl
- 450mL dH₂O
- 50mL Woolite

Purchasing Information

- Snap caps tubes (Falcon 5059)-VWR International
- Plastic rods (plastic inoculating loops)- VWR International
- Transfer pipettes- VWR International

Before Class

- Photocopy worksheet one and two for each set of students.
- Prepare water bath.
- Set up stations with appropriate material for each pair of students. This should include a rack, a permanent marker, soap buffer, ethanol, pureed fruit, a transfer pipet, a coffee filter, a plastic cup and a plastic rod.

During Class

- Review the cellular structures within plant cells, and discuss the similarities and differences between animal and plant cells.
- Ask students how they might extract DNA from eukaryotic cells, such as plant cells. They should realize after observing diagrams and discussing cellular structures that the outer membrane and wall are not the only barriers between a plant cell's DNA and the outside environment. There is also a nuclear membrane that has to be penetrated.
- Point out that breaking through a membrane can be compared to breaking through a wall. If a builder needed to knock down a wall, how would he or she determine which tools might work best? What specifically would the builder need to know about the wall itself?
- As a class, examine the components of the cell membrane. Make sure that the students understand that lipids are fat molecules. If a membrane is made primarily of fat molecules, what kind of tool could be used to break it down, or dissolve it?
- Discuss the fact that household soaps/detergents are used all the time to dissolve fat from greasy dishes, skin and laundry. In this experiment, dishwashing detergent dissolves the eukaryotic cell membrane and



nuclear membrane in the same way, thus releasing DNA from the cell.

- Read steps 1-4 aloud, demonstrate, and give students the opportunity to begin their experiments in small groups.
- During the incubation, discuss what is happening inside all of the tubes, and what will need to be done next. Once the DNA is released from the cells, it needs to be precipitated from the soapy solution in which it lies.
- Demonstrate how to use a coffee filter to separate the fruit pulp in the tube from the liquid cell contents. Collect the liquid in a clean cut and transfer it to an empty snap-cap tube.
- Read step 5, and draw a diagram of precipitation with ethanol. Make sure students understand that ethanol is a type of alcohol, similar to rubbing alcohol. Because of its chemical properties, DNA is not soluble in ethanol, and it can therefore be used to separate the DNA from the soapy solution in the tube.
- Demonstrate how to use the transfer pipette to add ethanol. Slowly pour ethanol down the side of a slanted bacteria tube and emphasize the importance of holding the tube on an angle.
- Show students how to spool the DNA from the ethanol layer with a plastic rod without breaking through the soap layer. It is essential that the two layers remain separated.
- If students would like to keep their DNA samples, they can be stored in small tubes of ethanol.

Analysis and Discussion

- Ask students how it was possible to see real DNA without the help of a microscope
- Discuss how James Watson and Francis Crick were able to determine the shape of the DNA molecule.
- Discuss the fact that the DNA extraction is an experiment that is not only used by scientists, but also by detectives (fingerprinting) and doctors (disease diagnosis). Why would someone want to extract *plant* DNA?
- Students are always curious to see if the double helix will be visible when magnified. If compound microscopes are available, spread some DNA on a slide, and stain with methylene blue. Even when magnified 400-1000X, the double helix will still not be visible, but students enjoy figuring this out on their own!

Further Explorations

Use a different protocol to extract DNA from eukaryotic cells, such as fruit. Compare and contrast the results.

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- Cells carry on the many functions needed to sustain life. They grow and divide, thereby producing more cells. This requires that they take in nutrients, which they use to provide energy for work that cells do and to make the materials that a cell or an organism needs.

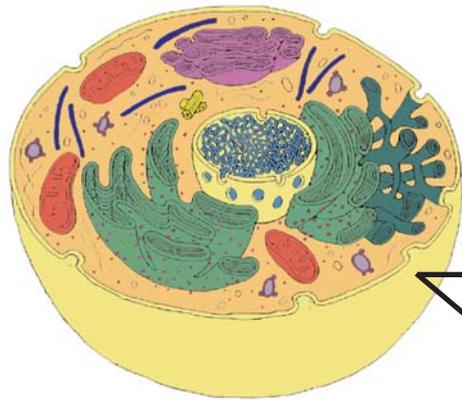
AAAS Benchmarks

Standard C: Cells

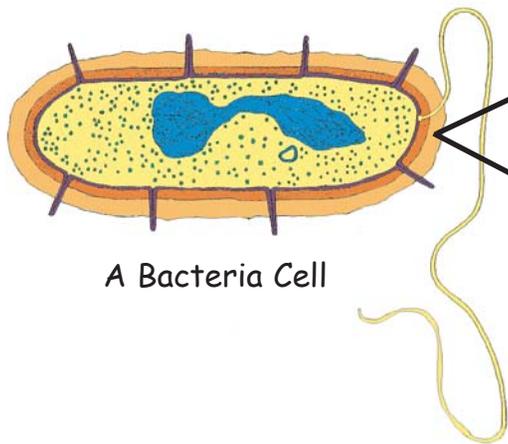
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- Microscopes make it possible to see that living things are made mostly of cells. Some organisms are made of a collection of similar cells that benefit from cooperating. Some organisms' cells vary greatly in appearance and perform very different roles in the organism.

THE CELL MEMBRANE

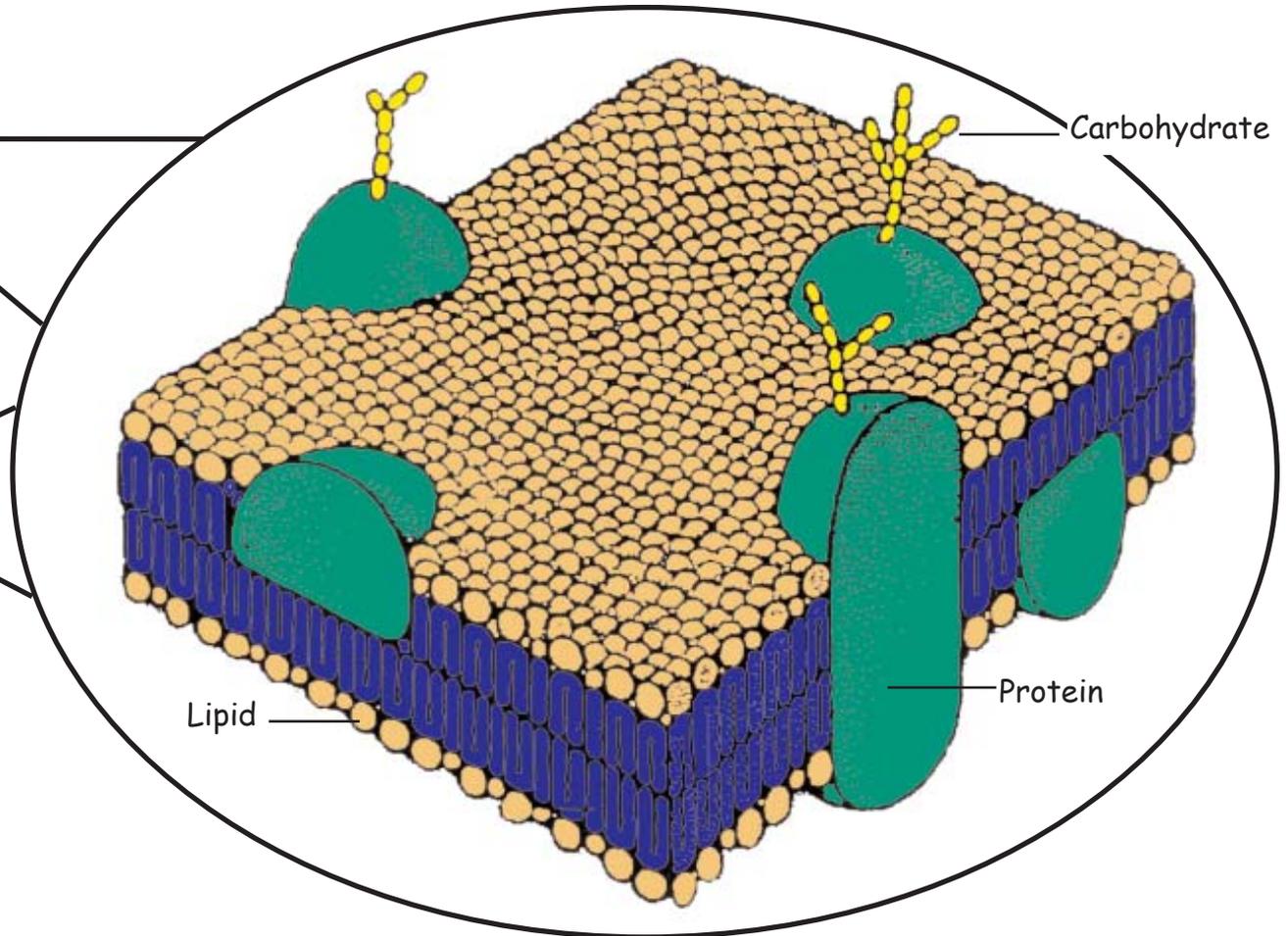
The cell membrane acts as a protective barrier which surrounds the cell. It is made of lipids, proteins and carbohydrates. There are two layers of lipids, one on the outside of the cell and one on the inside. The proteins are found throughout the lipid layer and control the movement of materials into and out of the cell. The carbohydrates are attached to the proteins on the outside of the membrane and help the cell to send and receive messages from other cells.



An Animal Cell



A Bacteria Cell



DNA EXTRACTION

1

Add **3mL** (milliliters) of soap to the tube of bacteria cells.

2

Shake to mix completely

3

Use the permanent marker in your rack to write your initials and your partner's initials on the white window on the side of the tube.

4

Place the tube in a **hot**, 65-75°C, water bath for **15 minutes**.



5

While holding your tube on an angle, use a dropper to carefully pour **3mL** of ethanol on top of the mixture of soap and cells.

6

Use a plastic loop to lift the DNA, floating in the ethanol layer, from the tube. Be careful not to push the plastic loop down into the soap layer.



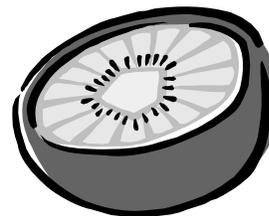
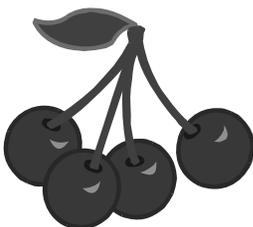
DNA Extraction From Plants



- 1 Pour 5 mL of soap buffer into a tube of well mashed fruit. Close the tube and shake well.
- 2 Label the tube with your partner's initials and your own.
- 3 Incubate the tube in a 60°C water bath for 5 - 15 minutes.
- 4 During the incubation, Partner 1 can get: a beaker full of ice and a tube of ice cold ethanol. Immediately place the ethanol tube in the beaker of ice.

Partner 2 can get: an empty beaker, a coffee filter, a clean 15 mL tube and a dropper.

- 5 Remove the reaction tube from the waterbath.
- 6 Carefully filter the fruit and soap solution through the coffee filter and into the empty beaker.
- 7 Pour the filtered fruit solution into the clean 15 mL tube.
- 8 While holding the tube on an angle, carefully drizzle 3 mL of ice cold ethanol on top of the mixture of soap and cells.
- 9 Let the reaction sit for 5 minutes and watch as a cloudy precipitate forms.
- 10 Use a glass rod to carefully spool the DNA from the ethanol layer.



Did the DNA from the plant cells look the same as or different from the DNA extracted from the bacteria cells?

What did the DNA look like under the microscope? Could you see the double helix structure?