EDVOTEK®

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Edvo-Kit #

S-75

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Do Onions, Strawberries and Bananas Have DNA?

Experiment Objective:

In this experiment, students will learn about the physical nature of DNA by isolating DNA from onions and using the common procedure of DNA spooling.

See page 3 for storage instructions.

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Table of Contents

Safety Data Sheets can be found on our website: **www.edvotek.com/safety-data-sheets**

Experiment Components

Contents	Check (✓)
• DNA Extraction Buffer	❑
• DNA sample in a capped test tube	❑
• Transfer pipets	❑
• Colored Beads (4 colors)	❑
• Graduated test tubes	❑
• Spooling rods	❑
• Salt packet	❑

Experiment #S-75 is designed for 10 groups.

Storage:
Store entire experiment in the refrigerator.

Requirements

- Fresh onion pieces (scallions, also called green onions, are highly recommended)
- Ripened fresh strawberry or banana pieces
- 20 ml beaker
- Test tubes (13 x 100 mm)
- 70% clear isopropyl alcohol (rubbing alcohol)
- Distilled water
- Ice

All experiment components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

Background Information

DNA - DEOXYRIBONUCLEASE

Since the 1800s, it has been known that 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 cells.

In 1868, a Swiss biologist named Friedrich Meischer carried out research which indicated that the nucleus of cells contains a material which he called nucleic acid. But, it wasn't until much later in the 1940's that the nucleic acid, deoxyribonucleic acid (DNA), was recognized as the carrier of genetic information.

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 generation to generation of cells. In its second important role, DNA provides instructions for making proteins, which are vital to the maintenance and function of cells. DNA provides information for the order of amino acids required for making various proteins.

The structure of the DNA molecule was determined by James Watson and Francis Crick in 1953. They determined that DNA was a double helix consisting of two strands. The Watson and Crick model is often described as a spiral ladder. The two strands of DNA are the backbone of the ladder, made of sugar phosphodiester groups. The sugar backbone acts as a support for the rungs of the ladder. These rungs are composed of the chemical bases Adenine, Guanine, Cytosine, and Thymine. The first letters of these bases, A,G,C, and T, are used by scientists 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 occurs on the opposite strand. Similarly, G and C are opposite DNA strands. The bases are held together by weak bonds which are shown as dashed lines in Figure 1.

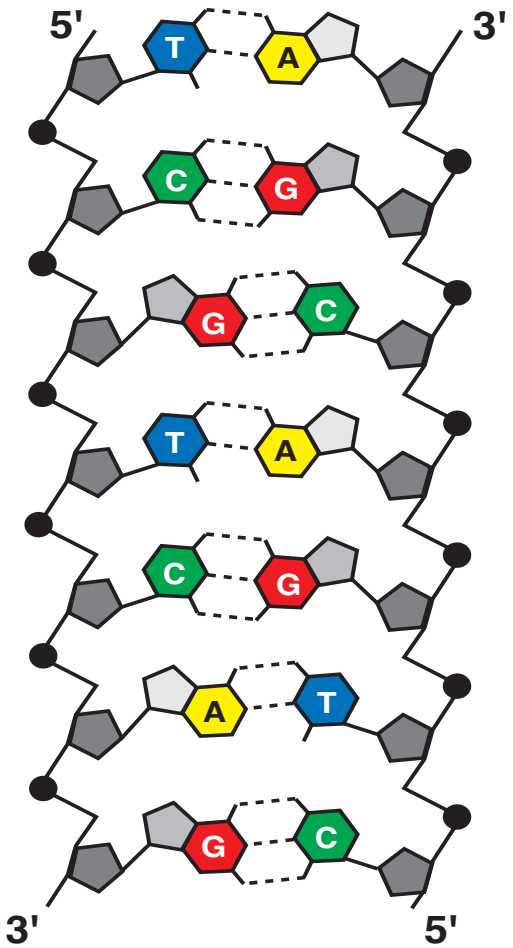


Figure 1: Structure of DNA

Background Information

DNA can be extracted from the nucleus of cells by adding an aqueous buffered extraction solution to cells. The cells are chemically lysed (broken open) and DNA from chromosomes is released. This procedure is known as cell lysis. DNA is soluble in water and cannot be seen, but it is insoluble in alcohol. Purification procedures for nucleic acids usually include precipitation with alcohol in the presence of salt. Since rubbing alcohol (isopropyl alcohol) has a lower density than water, it will form a second layer above the DNA solution. A spooling rod is used to spool the two liquids at the interface of the two phases to separate the DNA from the solution. The DNA will appear as a viscous, clotted mass as it is collected on the spooling rod (Figure 2). The amount of DNA spooled is a consequence of the size of the DNA fragments which are much larger than the small bio-molecules such as amino acids and small carbohydrate sugars.

In this experiment, DNA will be isolated in one of two ways: 1) DNA will be spooled from a buffer solution containing salts; 2) DNA will be extracted from tissues such as onion, banana, or strawberry and spooled from solution. DNA will be observed by the naked eye on the spooling rod.

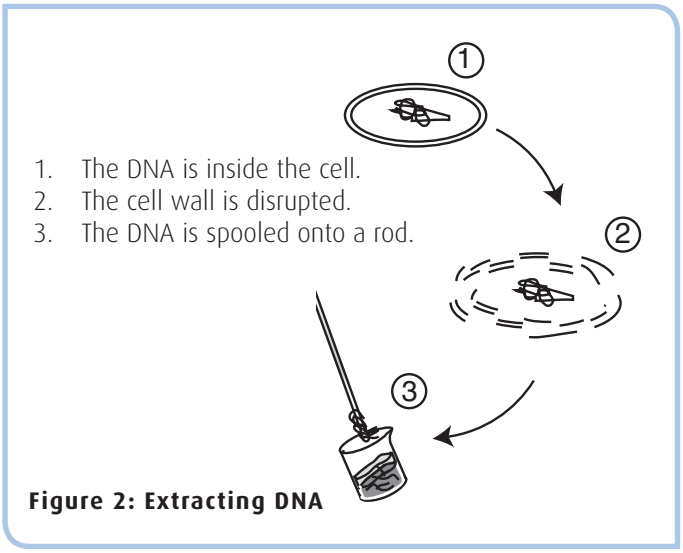


Figure 2: Extracting DNA

Experiment Overview

EXPERIMENT OBJECTIVE:

In this experiment, students will learn about the physical nature of DNA by isolating DNA from onions and using the common procedure of DNA spooling.

LABORATORY SAFETY

1. Gloves and goggles should be worn routinely as good laboratory practice.
2. Exercise extreme caution when working with equipment that is used in conjunction with the heating and/or melting of reagents.
3. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
4. Exercise caution when using any electrical equipment in the laboratory.
5. Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.

Wear gloves and safety goggles

WORKING HYPOTHESIS

DNA can be extracted from the cells, and isolated from solution by spooling.

LABORATORY NOTEBOOKS:

Scientists document everything that happens during an experiment, including experimental conditions, thoughts and observations while conducting the experiment, and, of course, any data collected. Today, you'll be documenting your experiment in a laboratory notebook or on a separate worksheet.

Before starting the Experiment:

- Carefully read the introduction and the protocol. Use this information to form a hypothesis for this experiment.
- Predict the results of your experiment.

During the Experiment:

- Record your observations.

After the Experiment:

- Interpret the results – does your data support or contradict your hypothesis?
- If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.

Activity One - Construction DNA Models

THE EXPERIMENT PROCEDURE

1. Each group of students will get a set of beads of various colors.

2. The teacher will assign a base designation for four of the colors. For example:

Red	=	Adenine (A)	Blue	=	Thymine (T)
Green	=	Guanine (G)	Yellow	=	Cytosine (C)

3. Sort all the beads by color. Based upon the beads you received, correctly put the colored beads together in two separate strands to form one or more of the following base pair models:

   A-T base pair G-C base pair

   How many A-T or G-C base pairs were you able to put together?

PUTTING ALL THE STUDENT BASE PAIR MODELS TOGETHER:

4. With the help of your teacher, and the rest of the class, build a model of the DNA ladder. Attach the ends of several bead sections together to form two long strands of double-stranded DNA.

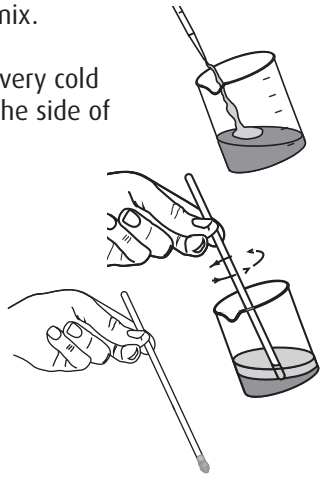
7. In your own words, describe the structure of DNA.

Activity Two - Teacher Demonstration of DNA Spooling

In this activity, DNA will be spooled from an aqueous buffered solution by adding salt and over laying with isopropyl alcohol. The isopropyl alcohol (clear rubbing alcohol) should be ice cold (stored in a freezer at least 2 hours before use). Just before the demonstration, place the DNA solution and the alcohol on ice.

THE CONTROL

1. Add two pinches of salt to a small beaker or clear graduated test tube.
2. Add distilled water up to the 2 ml level of the tube and swirl to dissolve the salt.
3. Use a transfer pipet to add 1 ml of the control solution (distilled water). Swirl to mix.
4. Using a transfer pipet, carefully overlay the control solution with two volumes of very cold 70% clear isopropyl alcohol (about 6 ml). Let the cold alcohol gently flow down the side of the beaker or test tube. Do not mix the two solutions.
5. Place the end of the spooling rod just below the line separating the two solutions (the interface). Twirl the rod with two fingers and spool.
6. Record your observations for the control.
7. Rinse the rod with distilled water and thoroughly dry with a paper towel.



THE EXPERIMENT

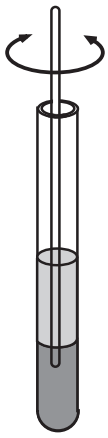
8. Add two pinches of salt to a small beaker or clear graduated test tube.
9. Use a transfer pipet to add 2 ml of distilled water and swirl to dissolve the salt.
10. Use a transfer pipet to transfer all of the DNA solution to the salt water. Stir or swirl to mix. The total volume should be approximately 3 ml.
11. Layer the cold alcohol on top of the DNA solution in the same manner as step #4 of the experiment. Do not mix the two solutions.
12. Place the end of the spooling rod just below the line separating the two solutions (the interface). Twirl the rod with two fingers and spool the DNA from the solution.
13. Observe the DNA and record your observations.

Wear gloves and safety goggles

Activity Three - Extraction of DNA from Onion Tissue

In this activity, DNA is extracted from onion tissue using an extraction buffer and spooled from solution by overlaying the liquid with ice cold isopropyl alcohol.

THE EXPERIMENT



1. Carefully slice a 5 x 5 x 5 mm cube (about the size of a pencil eraser) from the main body of the onion (not the root tip), and place it in one of the test tubes provided.

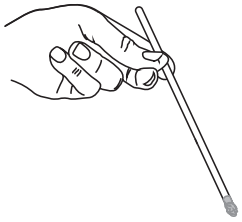
2. Using a transfer pipet, add DNA Extraction Buffer until it reaches the 3 ml level mark on the tube. Mince the onion cube with the tip of a spooling rod, pencil or pen (mash the onion very well). This will release the cellular contents and the DNA in the onion cells.

3. With a transfer pipet, carefully remove 2 ml of the liquid layer from the test tube and transfer it into a second, clean test tube. Try to minimize carry-over of the onion tissue.

4. Carefully overlay the liquid with 4 ml of **very cold** 70% clear Isopropyl alcohol.

5. Place a spooling rod into the test tube and twirl it with two of your fingers. Gently rotate the rod at the interface of the two phases. The DNA will begin to spool (wrap) around the rod.

   Gently lift the rod out of the solution from time to time and observe the DNA substance attached to it.



6. After spooling for several minutes, remove the rod from the test tube to observe the DNA. The DNA will appear as a viscous material adhering to the splint (it may be grayish in color if a pencil was used to mince the onion tissue).

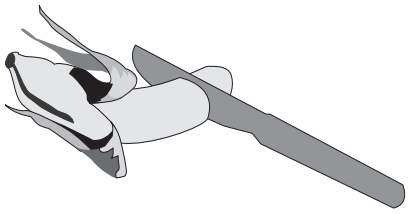
7. Record your observations of the onion DNA.

THE CONTROL

8. Based upon what you have previously learned, outline and perform the control for this activity.

Activity Four - Extraction of DNA from Strawberries or Banana

In this activity, DNA is extracted from a banana or strawberries in the same manner as the extraction of DNA from onion.



1. Carefully slice a 5 x 5 x 5 mm cube from the fleshy body of the banana or strawberry, and place it in a clean test tube.
2. Using a transfer pipet, add DNA Extraction Buffer until it reaches the 3 ml mark on the tube. Mince the cube with the tip of a spooling rod, pencil or pen to release the cellular contents.
3. With a transfer pipet, carefully remove 2 ml of the liquid layer from the test tube and transfer it into a second, clean test tube. Try to minimize carry-over of the banana or strawberry tissue.
4. Carefully overlay the liquid with 4 ml of **very cold** 70% clear Isopropyl alcohol.
5. Place a spooling rod into the test tube and twirl it with two of your fingers. Gently rotate the rod at the interface of the two phases. The DNA will begin to spool (wrap) around the rod.

   Gently lift the rod out of the solution from time to time and observe the DNA substance attached to it.
6. After spooling for several minutes, remove the rod from the test tube to observe the DNA. The DNA will appear as a viscous material adhering to the splint (it may be grayish in color if a pencil was used to mince the onion tissue).
7. Record your observations of the banana or strawberry DNA.

Study Questions

1. Describe the appearance of the isolated DNA.
2. What is a nucleus?
3. What are nucleotides?
4. What are chromosomes?
5. What does cell lysis mean?
6. Why did the alcohol layer on top of the DNA solution?
7. Why was it important not to mix the alcohol and DNA solutions?
8. What properties of DNA allow spooling?
9. What difficulties did you have in spooling? Why?
10. How many chromosomes do humans have?

Instructor's Guide

Pre-Lab Preparations:

ON THE DAY OF THE LAB:

1. Thoroughly chill the 70% isopropyl alcohol (rubbing alcohol) before the students perform the experiment.

   - Store it in the freezer for several hours or overnight to ensure that it is very cold.
   - Using a calibrated transfer pipet, transfer 6 ml of 70% isopropyl alcohol into 20 test tubes.
   - Place the alcohol-filled tubes back in the freezer or on ice until students are ready to use the alcohol.

Questions and Answers to Study Questions

1. **Describe the appearance of the isolated DNA.**

   DNA looks like a clear liquid gel. It turns white when it forms a precipitate.

2. **What is a nucleus?**

   The nucleus is an organelle present in a eukaryotic cell. It contains the chromosomes that are rich in DNA.

3. **What are nucleotides?**

   Nucleotides are the building blocks of DNA.

4. **What are chromosomes?**

   Chromosomes are inherited genetic units that are made of DNA.

5. **What does cell lysis mean?**

   Cell lysis is the disruption of a cell and cell nucleus with the release of the contents.

6. **Why did the alcohol layer on top of the DNA solution?**

   Alcohol is lower in density and therefore will "float" above the DNA in salt solution.

7. **Why was it important not to mix the alcohol and DNA solutions?**

   An interface is necessary in order to spool the DNA from the solution. If the aqueous layer containing DNA is mixed with the alcohol, DNA will precipitate out as a white fluffy product.

8. **What properties of DNA allow spooling?**

   The long length of DNA fragments makes it possible to spool it out of solution.

9. **What difficulties did you have in spooling? Why?**

   DNA may not have wound around the rod. Too much stirring may have resulted in fragmenting DNA into pieces too short to spool around the rod.

10. **How many chromosomes do humans have?**

    There are 46 chromosomes (23 pairs) in human cells.