EDVOTEK®

THE BIOTECHNOLOGY EDUCATION COMPANY®

Edvo-Kit #

S-10

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What Does DNA Look Like?

Experiment Objective:

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

See page 3 for storage instructions.

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

Experiment Components

Contents		Check (✓)
A	Concentrated Chromosomal DNA	❑
B	Concentrated Buffer	❑
•	InstaStain® Blue cards	❑
•	Transfer pipets	❑
•	Spooling rods	❑
•	Plastic disposable beakers	❑
•	Calibrated transfer pipets	❑

Experiment #S-10 is designed for 10 groups.

Storage:
Store entire experiment in the refrigerator.

Requirements *(not included with kit)*

- Pipet pumps or bulbs
- 50 or 100 ml graduated cylinder
- 20 ml beakers
- 100 ml beakers
- Test tubes (13 x 100 mm)
- 91% isopropyl alcohol (from a drugstore) or 95% ethanol
- Distilled water
- Paper towels
- Ice and ice buckets

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

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. Human cells contain a nucleus, which contains 46 chromosomes (23 pairs). Chromosomes contain DNA which encodes all the genetic information that is inherited from parents.

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.

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. 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 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 which are shown as dashed lines in Figure 1.

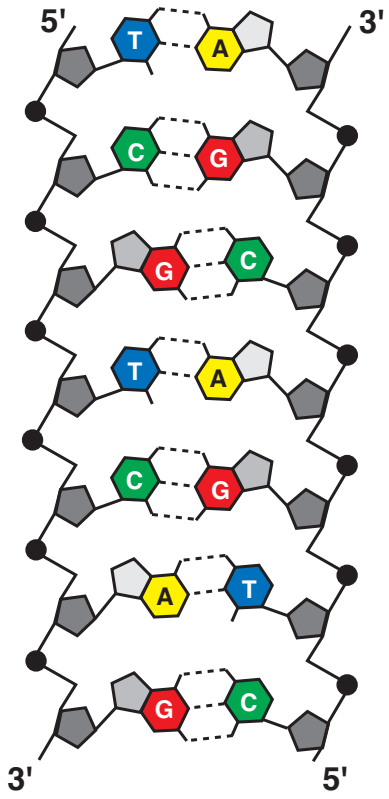


Figure 1 - Structure of DNA

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. DNA also 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.

When scientists study DNA, cells are chemically lysed (broken open) and DNA from chromosomes is released. This procedure is known as cell lysis. The solution of DNA is carefully overlaid with isopropanol. A spooling rod is used to mix the two liquids at their interface. The DNA precipitates from solution at the mixing zone and collects on the rod. This procedure is called isolation of chromosomal DNA and is frequently the first step in molecular cloning experiments.

DNA is soluble in water and thus will appear clear in water. But DNA is insoluble in alcohol, so it will form white fibers. Purification procedures for nucleic acids usually include precipitation with alcohol in the presence of salt. The solution of DNA is carefully overlaid with alcohol. 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

Background Information

the two liquids at their interface to separate the DNA from the solution (Figure 2). The DNA will appear as a viscous, clotted mass as it is collected on the spooling rod. 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. The precipitate can be redissolved in a smaller volume to concentrate it for further studies.

DNA spooling can, therefore, be viewed as a process that partially purifies and concentrates high molecular weight DNA. In this experiment, DNA will be spooled from a buffer solution containing salts.

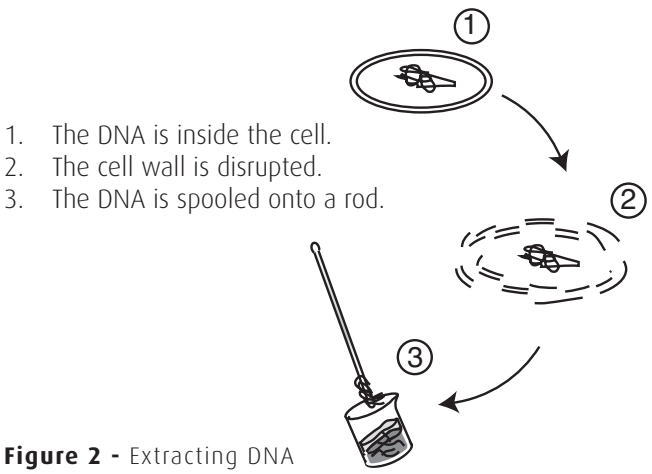


Figure 2 - Extracting DNA

Experiment Overview

EXPERIMENT OBJECTIVE:

In this experiment, students will learn about the physical nature of DNA by isolating DNA from a solution 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

If DNA is soluble in water and insoluble in alcohol, then overlaying a solution of DNA with alcohol and carefully mixing the two solutions with a spooling rod, should result in DNA precipitating out of solution and adhering to the rod.

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 - Spooling DNA from Solution

THE EXPERIMENT PROCEDURE

For Activity One, each student group should receive:

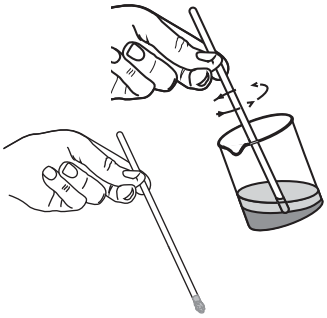
- Beaker labeled "DNA solution"
- Beaker Labeled "Control"
- 2 Test tubes, each containing 6 ml of very cold alcohol placed in a beaker filled with ice
- Transfer pipet
- One spooling rod
- Paper towel

Wear gloves and safety goggles

1. Obtain the materials for Activity One from your teacher. Be sure to keep the alcohol on ice to keep it cold.
2. Using a transfer pipet, carefully remove all of the alcohol from one of the test tubes.
3. Layer the alcohol on top of the control solution:
   - Place the tip of the pipet on the inside wall of the beaker.
   - Let the alcohol slowly stream down the side.

Note: Do not mix the two solutions!

4. Record your observations in your lab notebook or on a separate data form.
5. Place the end of the spooling rod just below the line separating the control solution from the alcohol (the interface).
6. Quickly twirl the rod in a circular motion.
7. After spooling (about 10 times), touch the rod to the bottom of the beaker and twirl it in place.
8. Remove the rod and allow the excess alcohol to drip onto paper towels. What do you see? Record your observations in your lab notebook or on a separate data form.
9. Rinse the rod with distilled water and thoroughly dry with a paper towel.
10. Using a transfer pipet, carefully remove all of the alcohol from the second testtube.
11. Layer the alcohol on top of the DNA solution:
    - Place the tip of the pipet on the inside wall of the beaker.
    - Let the alcohol slowly stream down the side.



Note: Do not mix the two solutions!

12. Record your observations in your lab notebook or on a separate data form.
13. Place the end of the spooling rod just below the line separating the DNA solution from the alcohol (the interface).
14. Quickly twirl the rod in a circular motion.
15. After spooling (about 10 times), touch the rod to the bottom of the beaker and twirl it in place.
16. Remove the rod and allow the excess alcohol to drip onto paper towels. What do you see? Record your observations in your lab notebook or on a separate data form.
17. Allow the DNA precipitate to air dry about 5 minutes. This will allow any remaining alcohol to evaporate before continuing with Activity Two.

Activity Two - Staining the Spooled DNA

1. To facilitate visualization, you can stain the spooled DNA. Make sure you do this on a stack of paper towels:
   - Using a transfer pipet, place approximately 10 drops of distilled water onto the InstaStain® Blue card to liquify the stain.
   - Transfer 2-3 drops of the blue liquified stain onto the DNA adhering to the spooling rod.

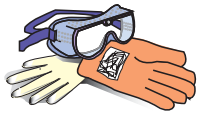
   Record Your Observations

2. Now add 2-3 drops of the liquified blue stain to the beaker from which you spooled the DNA. The stain will bind to residual DNA in the solution that did not spool onto the rod.
3. Now add 2-3 drops of the liquified stain to a beaker containing only water. Observe how the dye disperses in the water compared to the solution containing DNA.

   Record Your Observations.

For Activity Two, each student group should receive:

- InstaStain® Blue
- Beaker with distilled water
- Paper towels



Wear gloves and safety goggles

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

MATERIALS FOR THE EXPERIMENT

Each Lab Group should have the following materials:

Activity One

- Beaker labeled "DNA solution"
- Beaker Labeled "Control"
- 2 Test tubes, each containing 6 ml of very cold alcohol
- Transfer pipet
- One spooling rod
- Paper towel
- Ice and ice bucket

Activity Two

- InstaStain® Blue
- Beaker with distilled water
- Transfer pipet
- Paper towels

Pre-Lab Preparations:

ON THE DAY OF THE LAB:

1. Thoroughly chill the alcohol (either 91% isopropyl alcohol or 95% ethanol) before the students perform the experiment.
   - Store the alcohol in the freezer for several hours or overnight to ensure that it is very cold.
   - Using a calibrated transfer pipet, transfer 6 ml of alcohol into 20 test tubes.
   - Place the alcohol-filled tubes back in the freezer or on ice until students are ready to perform the experiment.
2. Prepare the DNA Solution
   - Using a graduated cylinder, measure 21 mL of distilled water. Place the water in a 100 ml beaker.
   - Add the entire contents of the bottle of concentrated buffer (B) into the beaker to create the diluted buffer solution.
   - Mix the solution.
   - Add the concentrated chromosomal DNA (A) to the diluted buffer solution and gently mix.
3. Dispense the DNA Solution and the Control Solutions. Use a calibrated pipet to
   - Transfer 3 ml of diluted DNA solution into 10 test tubes or small beakers. Label these "DNA Solution".
   - Transfer 3 ml of distilled water into 10 test tubes or small beakers. Label these "Control".
4. Keep the beakers in the refrigerator or on ice until students are ready to perform the experiment.
5. After students spool the DNA on the spooling rod, they will stain it with InstaStain® Blue. The stain will bind to the DNA and form a blue complex. Students will require a transfer pipet, one InstaStain® Blue Card, and distilled water

Quick Reference

	# test tubes or beakers	Volume
DNA Solution	10	3 mL per tube
Control Solution	10	3 mL per tube
Ice-cold Alcohol	20	6 mL per tube (12 mL total)

InstaStain® Blue stain is safe and non-toxic, but will stain skin and clothing. Students should wear gloves and handle with care.

Please refer to the kit insert for the Answers to Study Questions