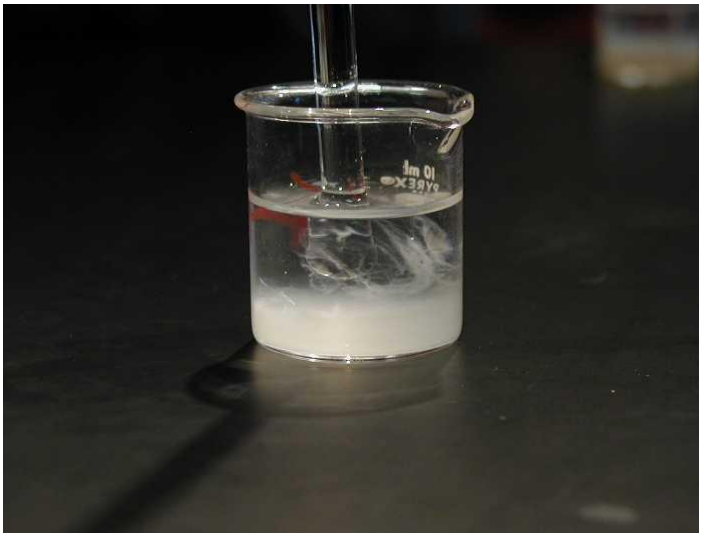
College of the Canyons
Biotechnology Program



DNA Extraction: VERSION B

How can DNA be used to solve crimes? Identify remains? Link family members?

DNA Spooling…it all starts here with the isolation and purification DNA from the other molecules in a cell. While it can be extracted from almost any living or preserved tissue, we will use bananas or another fruit, as they are easy to collect.

In this lab you will isolate DNA using common household chemicals. In a laboratory, many of the steps are similar, except more potent (and dangerous) chemicals are used in the process (chloroform and phenol for example).

Did you know that dollar for dollar, students taking biotechnology classes at community colleges have the most amount of money spent on their education? Grants for facilities, reagents, training, faculty etc. total in the hundreds of millions of dollars with only a few thousand students enrolled in biotechnology programs nation wide. Why? Simply put, the United States NEEDS trained biotechnology technicians to help run modern laboratories. So take more science classes!

Did you know that rigorous science training will make you more competitive for ANY type of job? Employers know that students who can tackle hard science will do well with almost ANY challenges presented them. For information on the biotechnology program and other robust science courses, contact: Jim Wolf, College of the Canyons Biotechnology Director at (661)362-3092 or email: jim.wolf@canyons.edu or visit the website: www.canyons.edu/users/wolfj
GOT SCIENCE? GET AHEAD!

LAB UNIT 5
DNA SPOOLING LAB
Version B

Objectives:

1. Extract and purify DNA from bananas or other fruits by spooling
2. Discuss the role of chemicals in the process of purification.
3. Examine the properties of DNA (base pairs, polarity, hydrogen bonding, and sequence structure)
4. Understand the central dogma of DNA: DNA $\rightarrow$ RNA $\rightarrow$ Protein $\rightarrow$ Trait

Background

Fifty years ago work with DNA was conducted mostly by Nobel laureate caliber researchers at prestigious universities (MIT, Cambridge, Stanford, USC). Today, DNA Technology is pervasive in the most unassuming environments. Produce at your local supermarkets has DNA technology that make fruits and vegetables resistant to degradation. Criminal investigation and prosecutions use DNA to identify a persons' with accuracy in the trillions. Health services can determine the probability of developing disease and in some cases an approximate life span of a patient once infected.

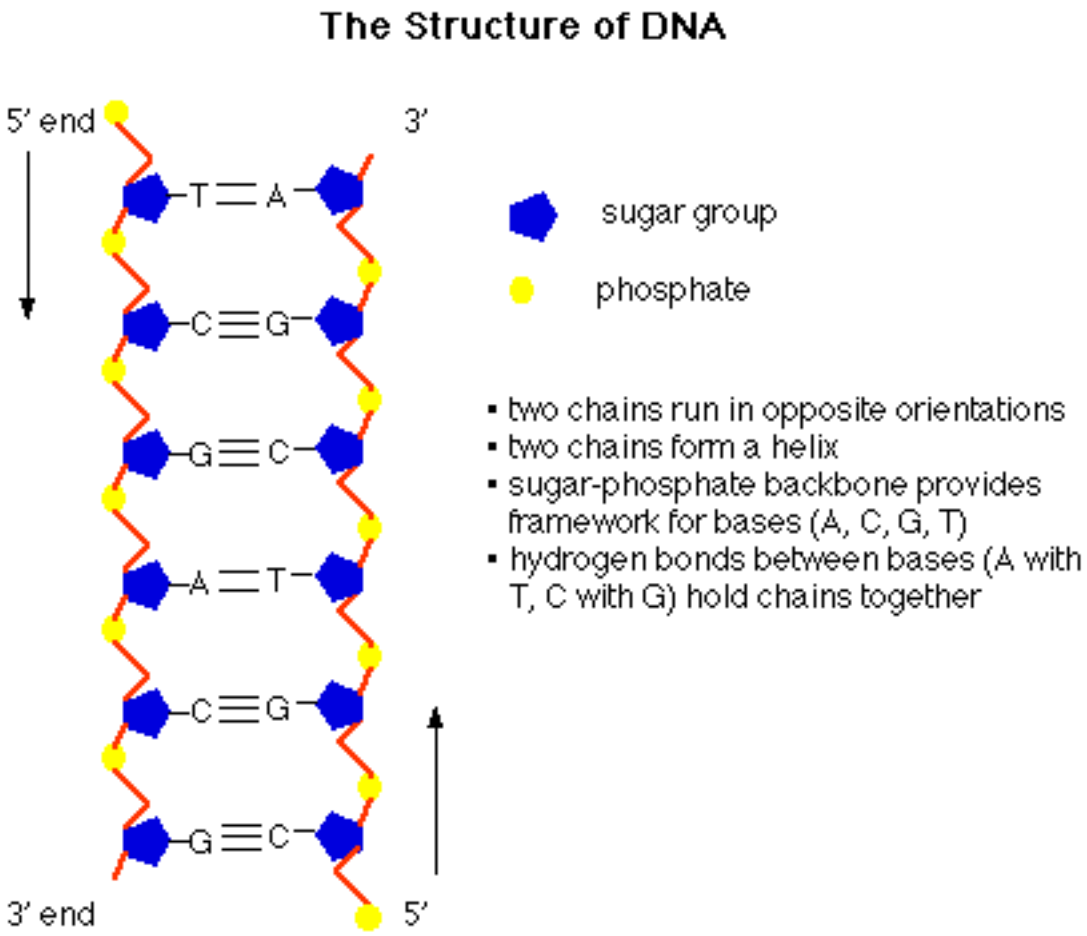
DNA is a macromolecule that is one of four that are necessary for cellular living including sugars, proteins, fats, and nucleic acids. DNA is a polymer composed of sugars and nucleic acids (Fig 1-DNA Diagram). They are linked two ways, by the covalent bonding of phosphates and sugars and the hydrogen binding of complementary base pairs of nucleic acids. DNA is the blueprint for cellular replication. The strands of DNA inside the nucleus are directions for creating all other components of the cell necessary for living.

The presence of DNA technology in commonplace settings exemplifies the need to understand the properties of DNA. The realized potential of DNA technology also dictates that scientists are in high demand in professional markets in the U.S. and across the globe. In fact, there are several biotechnology companies expanding in our own Santa Clarita Valley. COC's Biotechnology program is designed to provide students with the skill and knowledge to have an advantage in securing a job in this emerging field. So if you want to give yourself a competitive edge take science courses in high school and at College of the Canyons.

Overview

The steps in this laboratory procedure teach a great deal about the properties of cells, cell membranes, and deoxyribonucleic acid (DNA) itself. The collection of cheek cells from the inside of the mouth highlights the nature of body tissue. Dead cells are continually being sloughed off on both the inside and outside of the body. Recently sloughed cells still contain a nucleus and genetic material (DNA). This DNA can be collected and if in a forensics situation, analyzed and traced to a specific individual. Detergents solubilize and break down the lipids and proteins that form the primary cell membrane and disrupt the bonds that hold the membrane together. The cell contents, including the nucleus, are thus released and become available for further treatment or isolation. Sodium lauryl sulfate is an active ingredient in detergents. The final step requires the alcohol. The solubilized DNA comes in contact with the alcohol where the two liquid layers meet (called the interface).

The alcohol dehydrates and precipitates the DNA, as DNA is insoluble in the alcohol. If the procedure is done properly, fine, long strands of DNA will form at the interface and can be easily spooled onto a stirring rod.



Materials Spooling Lab
Version B Checklist

Work in pairs and collect the following items in a disposable tray and return to your lab station.

- ☐ 15mL clear polystrene test tube
- ☐ Stirring rod (wooden dowel)
- ☐ 1ml transfer pipet
- ☐ Dixie cup
- ☐ 0.8% NaCl solution in labeled squeeze bottle
- ☐ 1ml 25% liquid dishwashing detergent in 1.5mL Microfuge tubes
- ☐ 4-6ml 90% isopropanol alcohol in labeled bottle (not squeeze)
- ☐ Ice bucket
- ☐ 1ml TBE buffer solution

- ☐ PCR tube
- ☐ Nylon string
- ☐ Graduated cylinders
- ☐ Fruit cut into small slices
- ☐ Plastic sandwich bag

Procedure

I. Extraction of DNA

1. Place isopropanol on ice.

2. Fill Dixie cup half way (~5mL) with 0.8% NaCl solution.

3. Place a small amount of fruit into the plastic sandwich bag. Pour in the 0.8% NaCl solution into the bag.

4. Gently mash up the fruit with the saline solution for at least 30 seconds. Be careful not to spill the fruit solution out of the sandwich bag.

II. Releasing the DNA from inside the fruit cells.

1. Add 1 ml of the 25% liquid dishwashing detergent solution to the fruit mixture in the sandwich bag.

2. Gently mix the detergent solution throughout the fruit mixture. (The detergent removes the cell membranes from the fruit cells, releasing the DNA into the salt solution.) Wait 5 minutes.

3. Collect the fruit mixture into the bottom corner of the sandwich bag and hold the corner over the opening of the large test tube.

4. Tear or cut a small hole in the corner of the bag to allow the fruit solution to drip into the test tube.

III. Precipitate the DNA.

1. Holding the test tube at a slight angle carefully add 4 ml (about 2 full transfer pipet loads) of 90% isopropanol (ice cold) slowly down the side of the test tube so that it forms a layer over the "fruit cell" mixture in the test tube.

2. Hold the test tube upright for one minute and observe what happens at the interface between the isopropanol and the "fruit cell" solution. The cloud of white strands is the DNA. The DNA is not soluble in isopropyl alcohol, so it precipitates where the two liquids meet. Soap bubbles from the "fruit cell" solution will get trapped in the DNA strands.

IV. Collect the DNA.

1. Add 0.5ml of TBE buffer to the PCR tube.

2. Place a clean wooden dowel in the test tube containing the DNA. Collect the DNA by winding it on the rod by turning the rod in one direction. Discard the remaining solution into the sink and clean out tube for use by the next lab.

3. Carefully, remove the rod and DNA from the solution and transfer it to the PCR tube containing 0.5ml of TBE buffer. Observe the DNA strands floating in the buffer. OPTIONAL: ADD A DROP OF A DILUTE FOOD COLORING SOLUTION TO THE TUBE TO HIGHLIGHT YOUR DNA.

4. Make a bracelet or necklace as available for your class. Go forth and show the world your "Fruity" DNA.

Name_____

Questions

1. What is the purpose of each of the following components in this protocol?

A. Dishwashing liquid

B. Salt

C. Isopropanol

2. We can't really see a DNA molecule under the microscope unless it is tightly coiled into a chromosome. Why can you see the DNA after you put it into the isopropanol?

3. A. What causes the DNA to precipitate and spool on the rod?

B. Is this a single strand of DNA?

C. What structural characteristics of DNA allow it to be spooled out on a glass rod? Why is it not possible to spool out precipitated proteins? (Hint: Compare the relative lengths of DNA and protein molecules.)

4. Given this single strand of DNA provide the complementary strand creating a double strand of DNA. Include the correct base pairs, identify the number and types of bonds, label the 5' and 3' ends, and number of amino acids. 5'--- A T C C T T G A A G C T T G A C T A A T G G T C ---3'

# of H-bonds_____

# of covalent bonds_____

# of amino acids _____

5. The blueprint for cellular replication is _____ and is found within the nucleus. The process of transcription produces _____, which is single stranded. In the cytoplasm the process of translation occurs and produces _____, a string of polypeptides. During translation mRNA binds with tRNA via the hydrogen bonds of 3 complementary_____. The mRNA 3 complementary _____(previous answer) is called the _____, while the 3 complementary _____(previous answer) of the tRNA is called the _____. Each tRNA attaches only one _____.