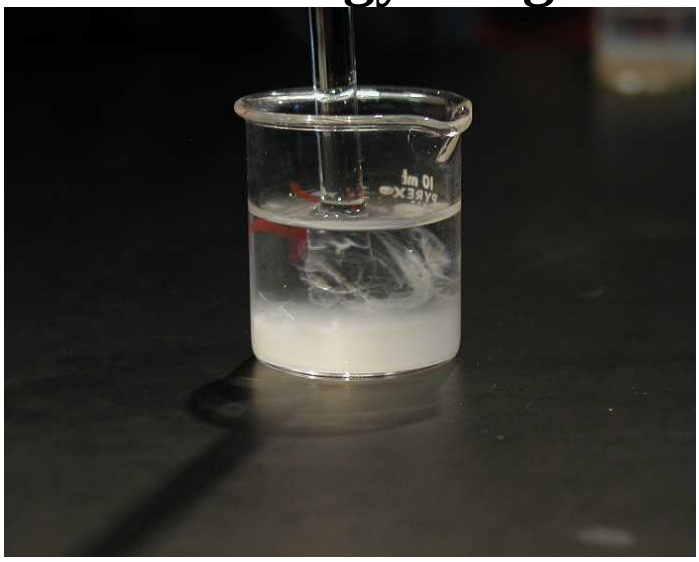
College of the Canyons
Biotechnology Program



DNA Extraction: VERSION A

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 the cell. While it can be extracted from almost any living or preserved tissue, we will use your cheek cells, as they are easy to collect (just rinse and spit!).

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 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 biotechnology and other robust science courses, contact: Jim Wolf, College of the Canyons Biotechnology Program Director at (661)362-3092 or email: jim.wolf@canyons.edu or visit our website: www.canyons.edu/users/wolfj
GOT SCIENCE? GET AHEAD!

LAB UNIT 5
DNA SPOOLING LAB
Version A

Objectives:

1. Prepare crucial solutions for purification of human DNA
2. Understand the role of reagents and steps required to isolate and rupture cheek cells and the role of alcohol to precipitate and purify DNA
3. Examine the properties of DNA (polarity, base pairs, hydrogen bonding, and sequence structure)
4. Understand the central dogma of DNA: DNA→ RNA → Protein → Trait
5. Utilize the structure of isolated DNA to spool on rod and use for a necklace to promote science.

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.

The expansive applications of DNA technology focus on the ability to isolate DNA from the nucleus of cells. In this laboratory you will isolate DNA from your own cheek cells. By simply swishing a saline solution in your mouth you will collect cheek cells that are naturally shed. You will then precipitate the DNA from the rest of the cellular components in the presence of an alcohol/detergent bilayer. Finally you will spool the DNA on a rod and place it into a vial to keep as a necklace. Now you can tell your friends that you have your own isolated DNA at your disposal. But beware, if it is lost it could turn up at a crime scene placed by an intelligent criminal or insurance companies can use it to raise your rates after identifying a "Lead-Foot" gene.

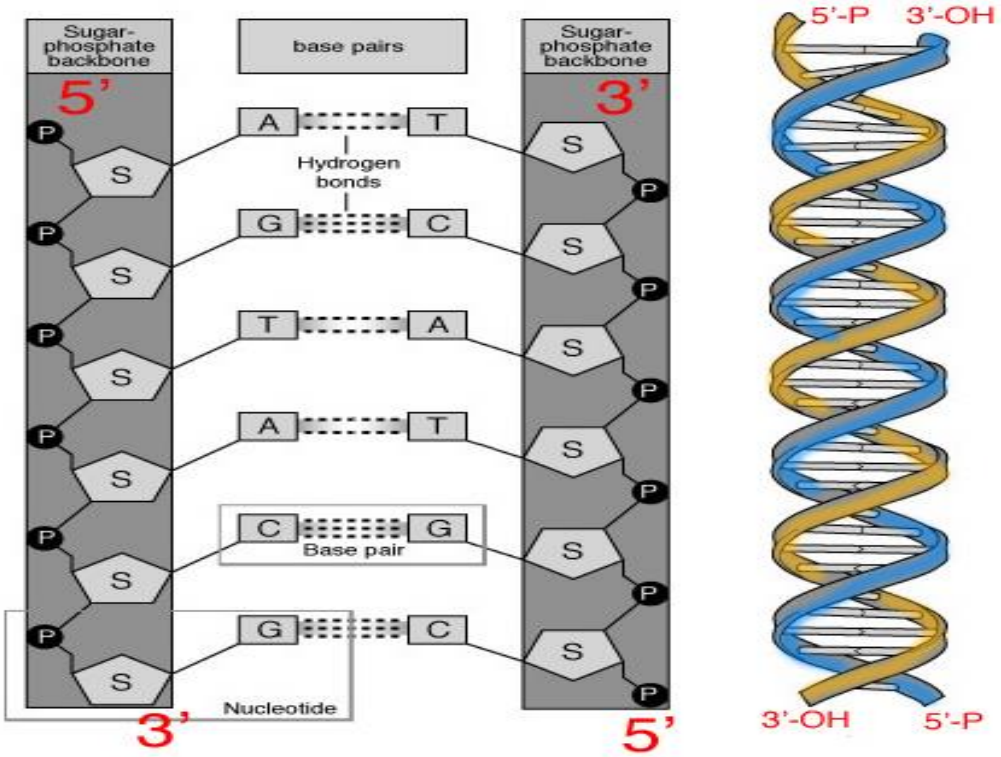
DNA is one of four macromolecules that are necessary for cellular living including sugars, proteins, fats, and nucleic acids. DNA is a polymer composed of sugars and nucleic acids. They are linked by covalent and hydrogen bonds (Fig 1 – DNA Diagram). DNA is the blueprint for cellular replication. The 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 many 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 it 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.



Spooling Lab
Version A Checklist

Work in pairs and collect the following items in a disposable tray and return to your lab station. If necessary use the calculations table on the handout to prepare solutions listed below.

- ☐ Stirring rod (wooden dowel)
- ☐ 1ml transfer pipet 2x
- ☐ 15mL clear polycarbonate test tube or falcon tube with lid.
- ☐ 0.8% NaCl solution squeeze bottle
- ☐ 1ml 25% liquid dishwashing detergent in 1.5mL Microfuge Tube
- ☐ 4-6 ml 90% isopropanol alcohol in 100mL bottle (not squeeze)
- ☐ Ice bucket
- ☐ 1ml TBE buffer solution
- ☐ PCR tube
- ☐ Nylon string
- ☐ Graduated cylinders

Procedure

I. Preparation

A. Check with the instructor for the amounts required for the whole class. Prepare as directed.

B. Prepare a 0.8% sodium chloride solution by dissolving 0.80g of sodium chloride in 99.2 ml of distilled water. Place solution in labeled squeeze bottle.

C. Prepare a 25% dishwashing detergent solution by mixing 25 ml of liquid detergent concentrate with 75 ml of distilled water. Subaliquot into 1.5mL Microfuge tubes using a 1mL Transfer pipette.

D. Prepare 90% isopropanol from 100% isopropanol: Mix 90mL of 100% isopropanol with 10mL of distilled water. Pour into a labeled bottle and place on ice.

II. Procedure

A. Fill Dixie cup half way with 0.8%NaCl.
B. Place the saline solution into your mouth and swirl the water around for at least one minute for an increased yield. Spit the water into the cup. Slightly bend cup to help in pouring contents into falcon or test tube. The swirling of the water washes cells from the inside of your cheeks into the water.

III. Releasing the DNA from inside the cheek cells

A. Add 1mL of the 25% liquid dishwashing detergent solution into the “cheek” mixture in the test tube.

B. Carefully stretch a piece of parafilm without tearing it around the opening of the test tube. Be sure no liquid can escape from the tube.
C. Mix the contents of the tube by gently inverting the test tube four times. **Do not shake the test tube** (the detergent removes the cell membranes from the cheek cells, releasing the DNA into the salt solution). Wait 5 minutes.

IV. Precipitate the DNA.

A. Holding the test tube at a slight angle carefully add 4ml (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 "cheek cell" mixture in the test tube.

B. Hold the test tube upright for one minute and observe what happens at the interface between the isopropanol and the "cheek 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 "cheek cell" solution will get trapped in the DNA strands.

V. Collect the DNA.

A. Add 0.5ml of TBE buffer solution to the PCR tube.

B. 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.

C. Carefully, remove the rod and DNA from the solution and transfer it to the PCR tube containing 0.5ml of TBE buffer solution. Observe the DNA strands floating in the buffer solution. (Optional: add a drop of dilute food coloring solution to the tube to highlight your DNA.) Discard the contents of the tube down the sink and place tube into bucket of "bleach water prepared by your instructor.

D. Make a necklace or bracelet as available for the class and show the world your personal DNA!

Name_____

Solutions Preparation:

90% Isopropanol solution: _____ml of 100% isopropanol + _____ml of distilled water.

8.0% Sodium chloride solution: _____g of sodium chloride + _____ml of distilled water.

25% Dishwashing detergent solution: _____ml of liquid detergent concentrate + _____ml of distilled water.

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 _____.