

DNA Extraction

Pre-visit Preparation

Welcome to the University of Maryland Biotechnology Institute's (UMBI) SciTech Education Program. We hope that these materials are useful and will help prepare your students for a unique and exciting "hands-on" lab experience. We encourage you to review these pre-visit materials. The teacher background sheets are designed to increase your understanding of this topic. Students will have a richer experience if you go over the pre-visit materials with your class before your visit. For more information regarding SciTech, visit our website: www.umbi.umd.edu/~scitech. We encourage you to make copies of the pre-visit information for students and review some of the fundamental concepts before you arrive.

Summary of Student Experience

After a guided inquiry about DNA and a review of background information with the SciTech staff, students will generate a list of questions about DNA and how it can be extracted from cells. Student investigations will take place in the SciTech Education Program Laboratory. Students will design experiments to determine the most effective method for extracting DNA from a variety of plant materials. Students will perform experiments with a partner to observe if DNA can be isolated from prepared samples of cells and from samples that they will prepare themselves using a variety of laboratory equipment. Upon request, a Center of Marine Biotechnology (COMB) scientist will discuss his or her research, personal science career path and respond to student questions about possible careers in science.

Tips for a Successful SciTech Experience

Review the pre-visit materials and bring along any materials that you have been using in class to discuss or discover the properties of DNA (workbooks, texts, fact sheets, etc). This may help the students make connections to classroom materials prior to your return to school.



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Experimental Design Background

Stating the Question

Every experiment begins with a question that the experiment will be designed to answer. Formulating this question is often a challenging part of setting up a research project. Your students will be building on a basic question about the most efficient system for extracting DNA from a variety of plant materials. The framework of this question is: *What is the most effective method to extract DNA from plant materials?*

Hypothesis

The clearest way to write a hypothesis is to use “if...then” statements. For example: If (fill in procedure) is effective in extracting DNA from plant cells then (fill in expected results). The most common hypothesis in research is the *null hypothesis*, which simply states that the variable or experimental situation being tested will exhibit no significant difference from the controls. For example, one null hypothesis could state, “There will be no difference in the amount of DNA extracted from cells that are crushed in a mortar and pestle compared to cells that are not crushed”.

Controls

When designing an experiment, it is important to plan ahead so that the method you are testing is compared against a standard. Students will be given a positive control (salmon sperm), which will serve as a standard source of DNA. To ensure that the chemicals alone don't react to produce something that looks like DNA, one of the samples will be a negative control (water).



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DNA Background

Since DNA is an essential molecule to all living things (with the exception of some viruses), it is not surprising that elaborate mechanisms have evolved to protect it. To extract DNA successfully, it is helpful to understand these protective mechanisms.

The simplest organisms, **prokaryotes**, which include bacteria, do not have the protection of a membrane-bound nucleus. Rather, the DNA clings to an in-folding of the inner cell membrane and is protected from invading viral DNA by restriction enzymes that cut foreign DNA into small pieces. Methyl groups that are attached to its DNA protect the host cell from its own defenses. Methyl groups prevent restriction enzymes from cutting DNA.

As organisms become more complex, so do the mechanisms that protect their DNA. *Eukaryotic* DNA is contained within a membrane-bound nucleus. Plants have additional protection from a cell wall. All eukaryotes have DNase enzymes in the cytoplasm that cut DNA. To produce spoolable DNA in the laboratory, it is necessary to denature the DNases before interrupting the nuclear membrane, often by using heat or pH changes. DNA is a relatively sturdy molecule but its tremendous length makes it prone to breaking once it is away from its protective environment. If the DNA is broken or sheared in too many places, it won't spool. It is important to be gentle in the last steps of DNA extraction and to avoid violent shaking or vortexing that will shear the DNA.

Historical Milestones

1869

Johann Friedrich Miescher identifies a weakly acidic substance of unknown function in the nuclei of human white blood cells. This substance will later be called deoxyribonucleic acid, or DNA.

1912

Physicist Sir William Henry Bragg and his son, Sir William Lawrence Bragg, discover that they can deduce the atomic structure of crystals from their X-ray diffraction patterns. This scientific tool will be key in helping Watson and Crick determine DNA's structure.

1924

Microscope studies using stains for DNA and protein show that both substances are present in chromosomes.

1928

Franklin Griffith, a British medical officer, discovers that genetic information can be transferred from heat-killed bacterial cells to live ones. This phenomenon, called transformation, provides the first evidence that the genetic material is a heat-stable chemical.

1944



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Oswald Avery, and his colleagues Maclyn McCarty and Colin MacLeod, identify Griffith's transforming agent as DNA. However, their discovery is greeted with skepticism, in part because many scientists still believe that DNA is too simple a molecule to be the genetic material.

1949

Erwin Chargaff, a biochemist, reports that DNA composition is species specific; that is, the amount of DNA and its nitrogenous bases varies from one species to another. In addition, Chargaff finds that the amount of adenine equals the amount of thymine, and the amount of guanine equals the amount of cytosine in DNA from every species.

1953

James Watson and Francis Crick discover the molecular structure of DNA.

1962

Francis Crick, James Watson, and Maurice Wilkins receive the Nobel Prize for determining the molecular structure of DNA.

(Historic milestones link: Access Excellence, www.gene.com/gene/research/biotechnology/significant-milestones.jsp)

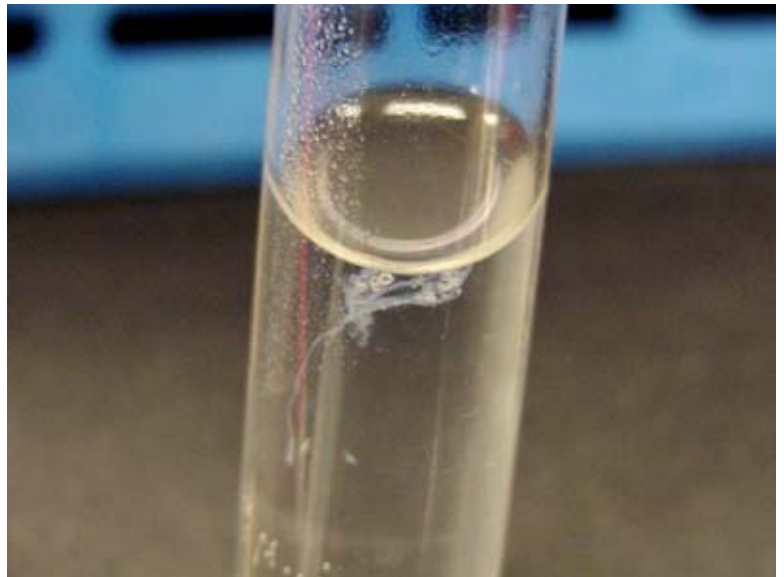
An Introduction to the DNA Extraction Lab

Some basic, but cool, chemistry...

DNA is the largest known molecule. A single unbroken strand can contain millions of atoms. When DNA is released from a cell it typically breaks up into tiny strand fragments. These tiny fragments have a slightly negative electric charge. Salt ions, common in many solutions, are attracted to the negative charges on the DNA fragments and prevent them from adhering to one another. By controlling the salt concentration of the solution containing the DNA fragments, DNA can remain fragmented or become very “sticky” and form large globs of molecular material.

Releasing the DNA...

The first step in obtaining DNA from any organism, be it a plant, animal, fungi, archae or bacterium, is to release the DNA from a cell. Detergents and soaps break down cell membranes, releasing the DNA, and they also break up proteins that may harm the DNA. Protein enzymes, or proteases, like those in contact lens solution or in “Ultra” forms of laundry detergent, can be used to further destroy proteins.



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DNA on a stick...

Once the DNA fragments are released into solution, the DNA can be spooled together by using ice-cold isopropyl alcohol. Alcohol allows DNA fragments to stick together, or precipitate, producing a blob of DNA that you can examine. When a small layer of alcohol is added to the top of a solution containing cellular fragments and DNA, it will form an interface where the DNA will precipitate, allowing it to be captured, or spooled, onto a wooden stick or glass rod. Although this method is effective, the DNA produced is by no means pure; other materials such as protein and cell fragments are carried along.

Student spoolers...

Following an introduction to DNA, students will have an opportunity to extract DNA from some interesting samples: salmon sperm and some common vegetables or fruits. Some samples will be prepared ahead of time (positive controls), but students will have to prepare other samples for themselves. The students will then compare their DNA extractions to positive samples. The steps are not complicated but do require that students work carefully to optimize the yield of DNA product that they are attempting to extract. The experiment will be a bit messy but very rewarding if students are able to make their own extraction.

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Glossary

Aliquot To dispense a specific amount of liquid.

Clone An exact genetic copy of an organism or a gene.

Deproteinization The process of removing proteins clinging to the surface of the DNA molecule and those found in the core of the DNA molecule.

DNA Genetic material found in all of our cells. DNA is an abbreviation for deoxyribo nucleic acid.

Lysing The process of breaking open cells.

Negative Control A sample with no DNA (e.g. water).

Positive Control A known source of DNA.

Precipitation The process of bringing compounds out of solution. DNA comes out of solution in alcohol, so visible DNA forms at the surface where the alcohol and cell sample meet.

Prokariotes Single-celled organisms, including bacteria, which do not have a nucleus.

Protease An enzyme that breaks down or denatures protein.

Protocol Set of directions for a lab procedure. Protocols are similar to recipes.

Restriction Enzymes Enzymes that cut DNA at specific base sequences.

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Book References

Carlson, Shawn. 1998. Spooling the Stuff of Life. *Scientific American, The Amateur Scientist*, September.

Horn, Toby M. 1993. *Working with DNA and Bacteria in Precollege Science Classrooms*. National Association of Biology Teachers, 11250 Roger Bacon Dr #19, Reston, VA, 22090.

Rasmussin and Matheson (eds). 1990. *A Sourcebook of Biotechnology Activities*. National Association of Biology Teachers, 11250 Roger Bacon Dr #19, Reston, VA, 22090.

Web References

Genentech - Biotechnology Activities:

<http://www.gene.com/gene/research/biotechnology>

DNA History and Facts:

http://www.genevue.com/A_DNA/History_2.html

DNA, Cells and Other Science Links:

<http://chroma.mbt.washington.edu/outreach/links.html>

Genetics Activities:

www.handsongenetics.com/summary.html

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Maryland State Department of Education Core Learning Goals

The following Core Learning Goals link directly to the SciTech Natural Products and Biosensors Lab. Take a few moments to review the specific goals, expectations, and indicators below so that you may prepare your students appropriately. If you do not have a Core Learning Goals document or CD, talk to your department chair, contact your science supervisor, or visit the website

http://www.mdk12.org/mspp/high_school/what_will/science/index.html.

Core Learning Goal- Science

Goal 1- Skills and Processes

Expectation 1.2 - The student will pose scientific questions and suggest investigative approaches to provide answers to questions.

Indicator 1.2.1 -

- The student will identify meaningful, answerable scientific questions.

Indicator 1.2.2 -

- The student will pose meaningful, answerable scientific questions.

Indicator 1.2.3 -

- The student will formulate a working hypothesis.

Indicator 1.2.4 -

- The student will test a working hypothesis.

Indicator 1.2.5 -

- The student will select appropriate instruments and materials to conduct an investigation

Expectation 1.3 - The student will carry out scientific investigations effectively and employ the instruments, systems of measurement, and materials of science appropriately.

Indicator 1.3.3 -

- The student will demonstrate safe handling of the chemicals and materials of science appropriately.

Indicator 1.3.4 -

- The student will learn the use of new instruments and equipment by following instructions in a manual or from oral direction.

Expectation 1.4 - The student will demonstrate that data analysis is a vital aspect of the processes of scientific inquiry and communication.

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Indicator 1.4.9 –

- The student will use analyzed data to confirm, modify, or reject a hypothesis.

Expectation 1.5 - The student will use the appropriate methods for communicating in writing and orally the processes and results of scientific investigation.

Indicator 1.5.1-

- The student will demonstrate the ability to summarize data (measurements/observations).

Indicator 1.5.2 -

- The student will explain scientific concepts and processes through drawing, writing, and/or oral communication.

Expectation 1.7 - The student will show that connections exist both within the various fields of science and among science and other disciplines including mathematics, social studies, language arts, fine arts, and technology.

Indicator 1.7.5 –

- The student will investigate career possibilities in the various areas of science.

Goal 3- Concepts of Biology

Expectation 3.1 - The student will be able to explain the correlation between the structure and function of biologically important molecules and their relationship to cell processes.

Indicator 3.1.1-

- The student will be able to describe the unique characteristics of chemical compounds and macromolecules utilized by living systems.

Expectation 3.3 - The student will analyze how traits are inherited and passed on from one generation to another.

Indicator 3.3.3 –

- The student will explain how a genetic trait is determined by the code in a DNA molecule.

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