

College of the Canyons' Biology Outreach Initiative:

High Performance Liquid Chromatography

Plasmid Insertion and Bacterial Selection

Size Exclusion Chromatography

DNA Finger Printing

DNA Spooling

Organic Macro-Molecules (OMM) *aka "the food lab"*

Program Includes:

6 Custom Made Modules

All Necessary Equipment

All Necessary Supplies

Workshops that Covers all of the Essentials

Technical Support

Teacher's Guides, Administrative Support, Computer Discs...and Much Much
MORE!!!

Including alignment of all modules with California Science Standards!!

**...ALL FREE TO INTERESTED HIGH SCHOOL
INSTRUCTORS AND STUDENTS IN THE
SANTA CLARITA VALLEY!!**

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SEE INCLUDED DESCRIPTION FOR DETAILS ON BIOLOGY MODULES.

College of the Canyons Biology Outreach Program Outline

The purpose of this outline is to provide the science instructor with a brief synopsis of the six modules included in the College of the Canyons Biology Outreach program. The first portion consists of a broad overview of the three distinct sections of the program. This will enable the instructor to review the themes and techniques employed and should facilitate both rapid assessment as well as aid in integrating these themes into a lecture format. The second section is a precise outline of the six modules citing: purpose, terms, apparatus, and/or mathematical concepts which the instructor may find useful in reviewing with their students prior to the lab exercise. Additional reference articles are cited at the end of each module (or in the teachers guide) and further advice and information may be attained by contacting Jim Wolf at the College of the Canyons (661) 362-3092 or visiting the biology outreach site @ <http://www.canyons.edu/host/biotechoutreach/> .

DESCRIPTION OF THE FOUR LABORATORY UNITS

UNIT I: (Modules 1, 2, 5, 6) SEPARATION AND IDENTIFICATION OF BIOLOGICAL MOLECULES

This lab unit will introduce students to a fundamental technique of modern biochemistry, the use of chromatography to separate complex mixtures of molecules. The unit includes two state-of-the-art chromatographic techniques. In module 1 students will use a Sep-Pak C₁₈ cartridge to cleanly separate the red and blue dyes in grape Kool-Aid. Methanol and isopropanol will be used as eluting solvents, and students will be asked to draw conclusions from the solvent's different separating properties. Module 2 will introduce size exclusion chromatography. A protein will be "desalted" using a Sephadex column. Modules 5 and 6 are recent modules designed to address the requests of local science faculty. Module 5 involves DNA isolation via a common spooling technique. DNA is extracted from either a student saliva sample or fruit, using dish soap and a salt solution. Ice-cold isopropanol is used to form a bi-layer and DNA is spooled from the interface between the two layers. Students can then place the DNA in a PCR tube and make a necklace or bracelet from their DNA sample. Module 6 involves the characterization of the major organic macromolecules (OMMs) in food. Five tests surveying topics of: calories, sugar, fats, proteins and starches are assessed after a student has made predictions based on nutritional analysis of various foods. A simplified version uses only three tests and leaves it up to the instructor to inform the student of the caloric and sugar content of their unknowns. This is a very robust lab that challenges students to make predictions and test their assertions while determining the identity of an unknown food item.

UNIT II: (Module 3) TRANSFORMATION OF E. coli

This experiment gives students experience in the manipulation of DNA and demonstrates DNA's ability to control cellular function. After a lesson on sterile microbiological techniques, students will manipulate bacterial cells to make them receptive to exogenous foreign DNA and then introduce a gene contained in a plasmid that will confer ampicillin resistance to cells which have received the gene. Both transformed and untreated cells will be grown on agar plates containing the antibiotic, so the difference in sensitivity to the antibiotic can be observed. Appropriate controls on agar plates without the antibiotic will also be made.

UNIT III: (Module 4) ELECTROPHORESIS AND DNA FINGERPRINTING

This unit provides an introduction to gel electrophoresis, a primary tool of molecular biology and medicine. The concept of DNA fingerprinting is also introduced. In the experiment designed for basic biology classes, the student is given an "unknown" sample of DNA that has been pre-digested by a restriction endonuclease. The student pours and loads an agarose gel and separates the DNA fragments electrophoretically. After staining with methylene blue, the pattern exhibited by the unknown DNA is identified by comparison with provided standards. In the AP level experiment, the student begins by digesting an "unknown" DNA sample with a series of restriction endonucleases. The resulting fragments are then separated by electrophoresis, stained, and identified. This experiment is set in the context of a hypothetical criminal investigation.

Specific Module Review

MODULE 1: HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

One of the central concepts in biochemistry is the idea of separation. The roles of separation cannot be overemphasized. Cellular components, chemical products, naturally extracted substances (from wheat germ to oil) all are mosaics of different compounds. To create more standardized and purer samples science employs a wide range of purification techniques. As complex as the purification equipment becomes they all revolve around a handful of purification techniques including filtration, distillation, dialysis and chromatography. High performance liquid chromatography exploits the differing solubility of substances to aid in their separation.

DEFINITIONS

- **Chromatography:** A method of chemical analysis based on the selective absorption of components of a mixture in a column of absorbent (column chromatography) or on a strip of paper (paper chromatography) etc.
- **Polarity:** A state or property of having poles. Usually associated with an unequal distribution of charge.
- **Solubility:** Capable of being dissolved or going into solution
- **van der Waal's forces:** Weak electrical interactions resulting primarily from temporary disparities in charge distribution. Van der Waal's forces are significant at close range and on larger molecules (especially DNA)

POINTS TO REVIEW:

- **Charges on particles:** Review the unequal distribution of electrons around polar bonds (C-N, C-O etc.) and comparatively equal distribution of electrons around non polar bonds (C-C and C-H).
- **Discuss the accumulation of charges** and relation to functional groups. Consider one polar bond on a small molecule as related to the same polar group on a larger molecule (reinforce the idea of charge to mass ratios). This point is central to the use of two different solutions to separate the "Kool- Aid". Tell students to focus on this idea as related to solubility.
- **Solubility:** Review ideas of like dissolving like (polar compound dissolving polar compounds and non-polar dissolving non polar).

MODULE 2: GEL FILTRATION

Often called size exclusion chromatography, gel filtration utilizes microscopic beads to conduct filtration on a molecular level. Technically this process is filtration (the sieving out of particles under pressure) however; its ability to separate led to the name size exclusion chromatography. The primarily physical (as opposed to chemical attraction in HPLC) method of separation and the striking size difference and properties of the particles separated makes this lab an excellent counter balance to HPLC. The effluent is clear which requires additional methods to "visualize" results. Conductivity checks for salt concentration and colorimetric tests for proteins allow for additional discussion and investigations into the science behind these detection techniques and assay techniques in general.

DEFINITIONS

- **Elution:** The process of drawing out substances based on solubility.
- **Colorimetric:** Assay techniques in which color is indicative of the concentration of a particular substance.

- **Desalting:** Process of removing salts, usually from a solution of proteins. Can be accomplished via dialysis, chromatography, gel filtration, and other methods.
- **Conductivity:** Ability to carry an electrical current. Water has poor conductivity but salt water has higher conductivity due to the presence of charged ions.

POINTS TO CONSIDER

- The role of the sieve should be discussed as the filter component that adds resolution.
- The idea of molecular size should be reviewed to reinforce the role of the beads' pore size in the process.
- The quantification of the effluent via conductivity (for salt concentration) and protein (via colorimetric assay) rarely equals the exact amount added to the column, suggesting that assay techniques (esp. colorimetric determinations which is VERY subjective) and labeling practices should be reviewed.

Mathematical consideration:

Students will prepare a graph of substance concentration (salt and protein) verse effluent volume. In addition to some data recording a review of graphing techniques are in order:

- Ensure that the axis are fully expanded (i.e. no graph tucked into the corner!).
- Ensure that unit spacing is uniform (the graph is linear / linear).
- Title and units are clear and conspicuous
- Remind students to draw best fit curve (using gently sloping lines that approximate a bell shaped curve) as a point to point graph does not reflect the variability inherent in the protocols.
- In addition the students should be asked to compare and contrast the nature of the two chromatographic techniques employed (provided that they are using both modules).
- AP students may use prepared graphs of elution volume verses molecular weight (MW) to predict the MW of an unknown protein (an exercise similar to this is included in the module).

MODULE 5: DNA SPOOLING

This lab is a good starting point for DNA analysis, as it requires only a modest amount of background science and a few supplies. DNA can be readily extracted either from the students' cheek cell's or from a wide range of fruits and vegetables. The soap readily ruptures key membranes and the DNA's low solubility in alcohol is used as the basis of adding a layer of alcohol to the top of the crude cell extract (AKA soap and cells). As the DNA precipitates out in the alcohol (due to its low solubility), the long linear molecules can be wrapped up on a spinning rod (hence the term spooling). Keep the isopropanol ICE COLD as this final step really impacts the yield of DNA.

DEFINITIONS:

Spooling: Processing of spinning a rod to collect long, linear DNA molecules as they catch on the rod's surface.

Precipitate: A physical process where a substance comes out of solution. Opposite of solubilize.

Interface: in chemistry speak, the layer that occurs between two solutions of different density. DNA will precipitate at the interface of the crude DNA extract (lower level) and the cold alcohol (upper level).

Detergent: A class of amphipathic molecules that help to dissolve items in solutions that they are not normally soluble in, such as in fats being dissolved in water.

Soluble: able to be dissolved into a solution. Opposite of precipitate in some respects.

POINTS TO REVIEW:

DNA's basic structure: As part of the handout, a review of DNA's basic structure is included. Review the sugar phosphate backbone and the 4 bases and principle of base pair. If you like, you can try the following question. A piece of DNA is 100 base pairs long and is 21% Adenine. From this simple piece of information the student can calculate the # of G,C, and Ts, purines, pyrimidines, sugars, phosphates and hydrogen bonds.

Solution chemistry and solubility. Solubility and precipitation should be reviewed and the need to keep a stable "bilayer" and its significance should be discussed. The soapy layer extracts the DNA from the cell and the alcohol precipitates the DNA from the soapy layer.

Role of extraction in other processes. This step of DNA extraction is often the first step in a number of later steps. From here the DNA may be cut up and analyzed (as in DNA finger printing) or amplified (via PCR) or continue on into many different directions as diverse as the field of biotechnology.

Mathematical considerations: This lab is relatively light in the math department as the lab is primarily a "proof of concept" lab showing that DNA is readily extracted from a range of cell samples. Version A has some simple percent solution calculations that the students make as they dilute solutions to the proper concentrations. There is also mention of "volume thirds" where students need to estimate the volume visually in a test tube and add 2 times the volume of a tube that is 1/3 full. This essentially tops off the tube with alcohol and helps to keep the volume cold (by the larger amount) during the follow-up spooling stage.

MODULE 6: ORGANIC MACROMOLECULES (OMMS), AKA THE FOOD LAB....

This lab is broadly used in one form or another by almost every high school biology and or chemistry department. It has a variety of permutations but most of the labs focus on using foods as a way of bridging the gap between the biology and chemistry. Students predict what the test results will be based on reviewing both the test specifics (i.e. how does the starch test work? the reducing sugar test?) and the nutritional content of various foods. Based on this information, students then predict their test results based on their unknown being one of four foods in a group. With this information in hand the students then carry out the tests; first with controls to help verify the test results, and then repeating these tests on a set of unknowns. This lab is very robust and challenging allowing students not only gain valuable technique pointers, but to also begin to understand the need for controls, and using the inductive and deductive reasoning processes as they relate to investigatory science,

DEFINITIONS:

Functional groups: small groups of atoms that impart key properties to a larger molecule.

Char test: used of extreme heat to burn (char) chemicals. In theory, molecules with calories will char (blacken), where as "non-calorie" foods (salt, baking soda, etc) will not char and will instead leave a white residue.

Organic carbon: carbon as found in foods that have calories. Will give a positive results for char test.

Calories: measure of extractable energy in foods.

Sugar: Class of organic macromolecules (OMM) that have carbon, hydrogen and oxygen in a ratio of 1:2:1. End in the letters ose and usually 3-8 carbons long and rich in hydroxyl functional groups.

Reducing sugar: A type of sugar with a free aldehyde. The permits the adjoining hydrogen to readily leave the sugar and land on another nearby molecule, hence reducing this molecule (and resulting in the oxidation of the sugar).

Hydrophobic: “water fearing” Molecules that have the property of being non-polar or otherwise dissolve poorly in water.

Hydrophilic: “water loving” Molecules that have the property of being polar or otherwise dissolve well in water.

Carbohydrate: class or molecules consisting on many monomer units of a sugars. Examples include starch, glycogen and cellulose. Iodine will complex with starch to turn bright blue.

Fat: An organic macro-molecules consisting of carbon, hydrogen and oxygen in a ratio of 1:2:less than one. The lack of oxygen makes these molecules hydrophobic. Steroids, phospholipids and triglycerides are the three primary classes of fats seen in cells and triglycerides are the “fat” see in most foods. Dissolves well in Sudan 4 dye (see below).

Reduction: chemical reaction where electrons and or hydrogen is passed to a another molecule. Call reduction as original work was done with metal ions and a copper ion (Cu^{+3}) is reduced (Cu^{+2}) when an electron is transfer (hence the copper is reduced).

Oxidation: Loss of electron and or proton. Often coupled with reduction action (see above) in what are called reduction/oxidation or “redox” reactions. Most reactions of significance in a cell are redox.

Protein: Polymer consisting of chain of amino acids. Amino group will react with blue copper ion in Biurets reagent to turn violet in color.

Amino acids: Monomer unit with amino and carboxylic acid functional groups. Come in 20 different types and are responsible for diverse range of protein functions.

Starch: Carbohydrate which is significant source of calories in many foods including bread, pasta and potatoes.

Biuret’s solution: copper based solution that reacts with blue copper ion to change color to violet when amino groups are present.

Benedict’s solution: Copper solution which can react with reducing sugar. Blue copper ion changes from Cu^{+3} to Cu^{+2} (is reduced) as sugar donates electrons to ion. Only sugars with a free aldehyde can accomplish this action (hence the term reducing sugar).

Sudan IV: Non polar dye that dissolve well in non polar media. Used as a test for fats that attract and hold brightly colored red Sudan particles.

POINTS TO REVIEW:

Test Review: Each test should be review prior to the lab. Cover both the *theory* as to what the test looks for and some *technique* issues. Concepts like going to open lab station (as opposed to going just in order of lab), using little reagent as possible, keeping things clean and labeled, go a long way to ensuring a quality lab experience.

Data Sheet: Controls: Review the sheet of positive and negative controls. Be sure to convey the need for this, as no self-respecting scientist would test unknowns using tests they are unfamiliar with.

Data Sheet: Unknown groups (A,B,C): The unknowns are grouped into three groups and a student knows that their unknown is one of the four in each group (A-C). Using this information and the nutritional analysis data, they will predict what each unknown food item will test like (inductive reasoning).

Data Sheet: Unknown Tests: When the time for the unknown tests actually arrives the students should be very prepared, having learned both the theory and practice of each of the tests! Each test eliminates a particular substance, and therefore the answer is deduced. This confirms another core idea in science in that it is easier to eliminate a substance that is it to identify. By eliminating all but one, the answer is deduced!.

Paperwork: Be sure to tell your students to complete all of the paperwork. Even if a single test clearly shows an answer (i.e. a negative char test could only be salt), they should complete all test and provide all supporting evidence. A answer without all supporting evidence is just a guess!

Mathematical concepts: The ratio of the elements can be used to help reinforce the difference between the major groups of OMMs. Additional analysis on the reactions and the related stoichiometry may be used as needed. This lab is relatively light in the mathematics though.

MODULE 3 TRANSFORMATION

The techniques involved in inserting DNA into bacteria and the subsequent selection and purification of products resulting from these bacteria has revolutionized science and the science classroom. This subject can lend itself to a wide range of additional topics from ethical to chemical and beyond. To keep the subject succinct it is best that the lecture conform specifically to the techniques and theory followed in the module (see previous lab review for synopsis) . The potential amount of background material is enormous however the following definitions and concepts have been shown to be pivotal.

DEFINITIONS:

Transformation: The process where by a cell takes in and uses a piece of DNA from its external environment.

Plasmid: Circular piece of DNA common among members of the bacteria.

Cloning: Reproduction by asexual division

Antibiotic: A substance in low concentrations which inhibits the growth of bacteria

Ampicillin: A common antibiotic similar to penicillin that interferes with the proper synthesis of components of the bacterial cell membrane.

Vector: A method of transport. A DNA molecule originating from one organism that can successfully enter the genome of another organism.

Recombinant DNA: Altered and or "recombined" DNA

***E. coli*:** Common bacteria: workhorse of genetic and biochemical manufacturing labs.

Luria broth. Growing media specific for some strains of bacteria

POINTS TO CONSIDER

- A discussion of the steps involved in protein synthesis from DNA to a protein will clarify the role of the plasmid in the creation of the final product.
- A discussion as to why bacteria make model organisms (asexual reproduction, fast growing, comparatively simple genome / biology, economical to grow) can be used to introduce the role of bacteria in recombinant DNA techniques.
- The role of antibiotic selection should be discussed as both a method to insure that the plasmid has been taken in and in an evolutionary / pathological context.
- Emphasis should be placed on the techniques involved and how the lab process can be very unforgiving to the sloppy or ill prepared. A clear understanding of the need for concise labeling and equipment / supplies identification is stressed (teamwork is critical in today's dynamic lab setting).
- The chemical (calcium chloride) and physical methods (heating) of disrupting the cell membrane to introduce the plasmid should be reviewed especially if the students are unfamiliar with the role and structure of the cell membrane.

MATHEMATICAL CONSIDERATION:

The exponential growth rate of bacteria can be simply expressed by: population number = 2^x where x shows the number of generations that have occurred. Assuming short turnover time and optimal survival bacteria can populate a given environment very quickly. Creating a graph of generation verses population number will show students the exponential growth rates of these organisms. This relationship can be visualized nicely by the following fact. Assume that a bacteria can divide every 20 minutes. If you start with a single bacterium, how big a blob of bacteria would exist after 96 hours? Assume that there are no limiting factors to the growth of bacteria. Answer: A blob the size of our solar system expanding outwards at the speed of light! (Essentially 2^{288}).

AP students can be additionally challenged by computing the efficiency of the transformation process. This is accomplished by quantifying DNA and bacteria numbers. Emphasis is placed upon word problems and unit conversion. See module for example exercise.

MODULE 4 DNA FINGERPRINTING

This module introduces DNA fingerprinting as well as the ubiquitous technique of electrophoresis. The separation of pieces of DNA into discreet bands that are characterized (fingerprinted) inextricably links this process to electrophoresis. The lab reinforces the separation motif that permeates biochemistry. It also forces the student to understand the process by investigating the unique structure of DNA. The role of endonucleases as specific cutting agents of DNA are reviewed in the context of a theoretical DNA investigation (criminal or paternal)

DEFINITIONS:

Endonuclease: Enzyme capable of cutting DNA at specific sites along a base sequence.

Electrophoresis: Migration of charged particles through a media under the influence of an electrical current.

Buffer: A pH stable solution often utilized to help organic macromolecules retain activity and / or shape in solution.

Agarose: Polysaccharide substance used as a gel (mesh) to separate chemicals as in electrophoresis.

POINTS TO REVIEW:

- The role of endonucleases should be discussed. Use two "demo pieces" of DNA to show the different sizes of the resulting fragments due to digestion by restriction enzyme will clarify this point.
- The subsequent fragments may be individual specific and upon separation show distinct banding patterns. The smaller fragments migrate further through the gel.
- The role of the electrical current, buffer, and agarose should be reviewed. Essentially they are as follows:
 - Agarose is the sieve through which the molecules are strained in addition to holding large amounts of water (thus permitting movement of molecules through it)
 - A buffer is incorporated to keep the DNA stable. While somewhat resilient DNA is still quite fragile and the role of buffers to retain natural state of the molecule should be discussed. Also the buffer aids in letting the electrical current flow (which would not occur in pure water).
 - The *electrical current* is a stream of electrons that bombards the particles of negatively charged DNA. As a result this stream of electrons nudges the DNA closer to the positive pole.

MATHEMATICAL CONSIDERATIONS:

Determination of DNA fragment length employs the use of standards as compared to unknowns. If a graph of migration distance verses DNA mass is prepared in order to make the resulting line

linear, a log / linear graph must be prepared. The weight of the DNA in log base₁₀ verses the distance migrated in linear units may be prepared to estimate the mass of an unknown fragment. This graphing technique is especially challenging and useful for AP students.