College of the Canyons'/(CLUES) Biotechnology Initiative

TOPICS:

Polymerase Chain Reaction
High Performance Liquid Chromatography
Plasmid Insertion and Bacterial Selection
Size Exclusion Chromatography
DNA Finger Printing

Program Includes:

5 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!!..

...ALL FREE TO INTERESTED HIGH SCHOOL AND COLLEGE INSTRUCTORS!!

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SEE INCLUDED DESCRIPTION FOR DETAILS ON BIOTECHNOLOGY MODULES.

College of the Canyons (CLUES) Program Outline.

The purpose of this outline is to provide the science instructor with a brief synopsis of the five modules included in the College of the Canyons CLUES biotechnology program. The first portion consists of a broad overview of the four 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 five 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 and further advice and information may be attained by contacting Jim Wolf at the College of the Canyons (661) 259-7800 ext. 3092.

DESCRIPTION OF THE FOUR LABORATORY UNITS

UNIT I: (Modules 1 and 2) 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 the first experiment students will use a Sep-Pak C_{18} cartridge to cleanly separate the red and blue dyes in grape KoolAid. Methanol and isopropanol will be used as eluting solvents, and students will be asked to draw conclusions from the solvent's different separating properties. The second experiment will introduce exclusion chromatography. A protein will be "desalted" using a Sephadex column.

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

This experiment gives students experience in the manipulation of DNA and demonstrates the DNA's ability to control cellular function. After a lesson on sterile micro biological 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.

UNIT IV: (Module 5) AMPLIFICATION OF DNA - THE POLYMERASE CHAIN REACTION

The culmination of the four-part laboratory curriculum, this unit will teach the students the theory and techniques behind the polymerase chain reaction (PCR), one of the most revolutionary technologies in molecular biology in the last decade. Students are given a "mystery gene" to amplify by hand cycling through ten cycles. Following amplification, the product DNA will be identified by running it on an electrophoresis gel and comparing it with provided standards. A hypothetical genetic counseling dilemma is presented in this lab in which students are asked to determine if their gene came from a normal individual or a carrier for a genetic disease.

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 can not 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 colormetric assay) suggests that assay techniques 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:
- Insure that the axis are fully expanded (i.e. no graph tucked into the corner!).
- Insure 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 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 generations 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 the 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 $base_{10}$ 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.

MODULE 5 POLYMERASE CHAIN REACTION (PCR)

This module has numerous applications in the sciences including forensics, genealogy, diagnosis of diseases and other applications where large amounts of DNA are needed from small or degraded samples. The process of accelerated replication via PCR allows rapid amplification of an undetectable

amount of DNA up to identifiable amounts using electrophoresis and staining. The technique is made more investigative in the context of real genetic questions regarding testing for a fatal disease within a family.

DEFINITIONS

- **Polymerase**: Enzyme involved in the replication of DNA by connecting the nucleotide bases into a polymer (DNA)
- ***Thermus aquaticus*** Species of bacteria from which a heat stable form of polymerase is isolated.
- **Templates**: The base pairing idea of DNA creates complimentary strands from initial templates of single stranded DNA
- **Annealing** : Process where by lowered temperature permits the rebonding of the individual strands of DNA into a two stranded helix via van der Wall's
(weak attractive) forces.
- **Primers**: Specific pieces / sequences of DNA that promote replication.
- **Homozygous**: Genetic condition where the individual has two *similar* forms of a gene (allele) on homologous chromosomes.
- **Heterozygous**: Genetic condition where the individual has two *dissimilar* forms of a gene (allele) on homologous chromosomes.

CONCEPTS

- This is a culminating lab that includes principles relating to Mendelian genetics, DNA replication, electrophoresis, and human genetics.
- The base pairing ideas of DNA as well as the mechanics of replication should be discussed. Specifically address the unzipping of the two-stranded DNA, the priming of the DNA and the rapid introduction of the complementary bases during replication. Note temperatures involved in these steps.
- The focus on the steps involved in thermal cycling need to be closely and precisely controlled. Have the students visualize the steps of unzipping, annealing, replication... back to unzipping etc. as related to temperature
- Module 4 serves as good primer for electrophoresis discussion.

MATHEMATICAL CONCEPTS:

- The replication rate of DNA can be expressed as DNA strands = 2^x where x equals the number of cycles completed. The idea of limiting compounds can be shown by discussing how long the replication can occur until supplies run out.