This is to certify that the

thesis entitled

DEVELOPING BIOTECHNOLOGY AND PLANT TECHNOLOGY
LABORATORY SKILLS AND ATTITUDES
IN HIGH SCHOOL STUDENTS

presented by

Robert D. Eicher

has been accepted towards fulfillment
of the requirements for

MS degree in Interdepartmental Biological Science

Major professor

Date 22 Nov 96

DEVELOPING BIOTECHNOLOGY AND PLANT TECHNOLOGY LABORATORY SKILLS AND ATTITUDES IN HIGH SCHOOL STUDENTS

By

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Interdepartmental Biological Sciences

1996

ABSTRACT

DEVELOPING BIOTECHNOLOGY AND PLANT TECHNOLOGY LABORATORY SKILLS AND ATTITUDES IN HIGH SCHOOL STUDENTS

By

Robert D. Eicher

The problem addressed in this thesis is two-fold. First, can it be expected that high school students learn to use and apply the lab techniques employed in biotechnological laboratories? Secondly, can it be shown that high school students show positive and objective attitudes towards plants and biotechnical research as a result of high school curriculum exposure?

Activities for this thesis were developed to be inserted into the existing curriculum over the course of the school year for students enrolled in Advanced Placement Biology. The activities include the developing of the following techniques; buffer calculation and preparation, spectrophotometry, column and paper chromatography, micropipetting, protein concentration analysis, aseptic technique and tissue culturing.

Significant gains in student confidence in all identified skill areas was evident in the exit survey results, as was the measure of the student attitudes towards biotechnology.

ACKNOWLEDGMENTS

Many people have provided assistance and encouragement during the years since I began this endeavor. I would like to expressed my gratitude to the following persons for whom without their guidance, patience and prodding, the completion of my degree would not have been realized:

The masters committee: Dr. Merle Heidemann for her unconditional assistance in the final year of my work, Dr. Clarence Suelter for his enthusiasm and direction in rekindling my desire to be a better biology teacher, and to Dr. Martin Hetherington and Dr. Howard Hagerman for their efforts in providing a great experience in environmental education at Kellogg Biological Station.

My colleagues that I shared three summers of science, fun and sweat with at MSU. There are many names and faces in my memories, but special thanks to my friends and mentors Eric Rittenhouse and Rich (Flash) Feezle.

My classroom students who have made these years of teaching go by quickly because of their eagerness to learn and their acceptance of my teaching style.

My children: Analiese, Aaron and Zachary, who have had to endure and give up their father on weekends and summer days.

My wife, Sue Ann Eicher, who watched me drive away from our home to MSU on our fifth wedding anniversary, my first Father's Day with our then three month old daughter Ana. Without whose encouragement, love and belief in me this journey would have never, ever have been possible.

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CHAPTER ONE

INTRODUCTION

It has been well established that "hands-on" and "minds-on" science education is of much greater value to a high school student than learning from a book or lecture format (National Science Education Standards {NSES}, 1996 p.20). A 'hands-on/minds-on' approach to teaching science provides a greater likelihood that the student will learn, retain and think about science on a higher level than without the use of such activities (NSES 1996, Cole 1990 New York Times Education Supplement p. 19). The way that we as educators approach laboratory activities must also undergo a renaissance if we are to effect a change in the way our students view science. "If students themselves participate in scientific investigations that progressively approximate good science, then the picture that they will come away with will likely be reasonably accurate" (Benchmarks For Science Literacy, 1993 p. 9). For many years teachers have focused heavily on covering content and memorizing factual knowledge. As the role of science education is being redefined we must not only look at what students are to learn, but also how it is being taught (Science For All Americans, 1990).

The goals of this document are commensurate with this approach of having the student assess the problem at hand and suggest a problem solving design that will attempt to answer the question. The students need to predict, collect data and analyze their findings reinforcing the process of scientific inquiry, especially as they apply to the lab skills involving biotechnology (the

application of the principles of engineering and technology to the life sciences). The central goals of this document are:

1. High school students can learn to use and apply laboratory skills and techniques that are employed in biotechnology:
   A. Chromatography
   B. Spectrophotometry
   C. Pipetting/Micropipetting
   D. Aseptic Technique
2. Students will be more likely to recognize plants as a biotechnological tool after the completion of the activities described within this document.
3. Students perspectives and attitudes toward biotechnology will be more positive after the completion of the activities described within this document.

The activities that are described within this document are designed to supplement any high school biology curriculum materials and can be inserted or utilized in their program where the individual teacher determines what will best suit the educational needs of their students. The materials and activities that are described within this text are commensurate with the science curriculum overseen by the Wisconsin State Department of Public Instruction. They particularly address the objectives stated for Science-Technology-Society (STS); "The aim of science education...is to develop scientific and technological literacy for all citizens." (STS: Science Education for the 1980's, 1982) which have been endorsed by both school districts that I have worked in during the last decade. The STS curriculum goals were not being addressed previously, before the implementation of the activities listed here. The skills that are required to perform the activities described are also skills that are needed for

the execution of laboratories suggested by The College Board for the Advanced Placement Biology Course in which these activities have been taught.

Why did I choose to focus on developing activities in biotechnology and plant biotechnology for my high school students? Using plants as a biotechnology tool has several advantages. First, plants do not run away or bite, therefore they are easily handled and manipulated in the classroom. Secondly, even though bacteria or mold many times provide a better laboratory vehicle, they are often viewed by students (and many teachers) as dirty, disease causing and potentially hazardous. Plants are seldom viewed in this same negative light, and therefore, are a great avenue for introducing skeptics to biotechnology with an innocuous organism that they can feel safe in handling. The transition to other biotechnological vehicles can be made more easily at another time if so desired.

The activities described in this document are performed in cooperative learning groups and present new lab techniques, inquiries and materials compared to what I previously taught. The techniques being described for use are not new to industry or research labs, but are activities and techniques that are not being utilized in the classroom. Students will benefit from this experience and be better prepared to compete academically and technologically for higher education and employment opportunities after the completion of high school.

The field of biology has changed dramatically in the past ten to fifteen years. Science teachers themselves need to return to the classroom so they can better train and teach their students. The activities described in this document are ones that can easily be adapted as a whole or individually into the teacher's curriculum. As the teacher becomes more familiar with newer classroom technology, more of the material may be readily usable and/or

adaptable for the teacher's own curricular situation. In terms of course curriculum, these activities are intended to employ biotechnology and plant biotechnology as a learning tool utilized as common technological thread throughout the curricular scope and sequence. The intention is to also make plants a laboratory choice considered by a teacher more often when an organism is needed to learn a concept in the classroom.

The activities were selected for their relevance to technology and/or their adaptability to utilize plant tissue. The activities described involve general knowledge about how to 'get around' in a laboratory, making solutions, adjusting pH, pipetting, measuring, mixing and storing. It will be assumed that most of the general laboratory technique that would be required is already in the repertoire of the teacher running the lab.

The evaluation of these activities was based on an exit survey consisting of two parts, and comparing two groups of students. The first part of the survey involves the level of student confidence in their lab skills developed as a result of class participation. Part two involves the students' attitudes regarding biotechnology and their own science education as a result of class participation. Group one students participated in the class during the school year 1994-1995, group two students participated during the school year 1995-1996. Both groups received the survey during June 1996. The data collected from the group one students will also serve as a measure of how the student impressions of their lab skills and attitudes toward biotechnology and science education have been retained over a period of one year.

In comparing an "old" approach to a "new" approach, there exists little to compare. Until I began participating in summer workshops, particularly the MSU/NSF Molecular Biology Summer Institutes at Michigan State University and Kellogg Biological Field Station, there was no "old" approach! I did not

teach the type of activities that are described in this document, much less have any training in the implementation of activities that addressed the objectives stated herein. The impetus for this change was founded in my desire to teach an advanced biology course. Since I had no previous experience in teaching this level of biology, I cannot compare the results to what I might have previously taught. I had been teaching introductory biology for several years prior to the onset of teaching advanced biology. The subject of botany often got shuffled aside in the haste of trying to manage covering the curriculum. Botany was whittled down to photosynthesis, distinguishing plant tissue from animal tissue and perhaps a leaf collection focusing on taxonomy.

LITERATURE REVIEW

We live in an increasingly technological society that will require that our citizenry be literate in the application of the sciences to their everyday world. Increasingly, biotechnology is playing a role in the current and future well being of the global population. "Throughout history, people have concerned themselves with the transmission of shared values, attitudes, and skills from one generation to the next." (Science For All Americans, Project 2061, 1990 p. 183). How we, as biology teachers, prepare our students to intelligently face decisions surrounding biotechnology in their daily existence may impact the progress of the scientific community in addressing problems that require support (both financial and political) from the lay population. For many students introductory biology will be the final science course taken in their formal education.

The potential impact of biotechnical education has not escaped the notice of the legislative branch of society empowered to enact laws that impact our lives and environment. In 1988 Gordon Meeks Jr. commenting on the National Conference of State Legislatures writes:

> The social and economic benefits of biotechnology...will undoubtedly touch every American. State involvement has been limited so far to development and promotion. Biotechnology is one of the nation's emerging industries and many see it as a bonanza for economic development. But as the industry grows, legislators increasingly will be expected to regulate its risks as well as support its economic potential.

The global community, including those elected to positions where decision making occurs, need to be knowledgeable and comfortable with the progress and proposals being pursued in the ever changing arena of biotechnology. The very legislators who will impact the progress and advancement of biotechnology in the political arena are, or will be, the product of our science classrooms. Science educators must ask themselves, will the product be technologically literate or not?

In 1986, Kenneth W. Dowling, Supervisor of Science Education with the state of Wisconsin Department of Public Instruction, submitted a working definition of science :

> Science is a human activity through which problems and questions dealing with natural phenomena can be identified and defined, and solutions proposed and tested. In this process data are collected and analyzed, and available knowledge is applied to explaining the results. Through this activity, investigators add to the store of knowledge, thereby helping people better understand their surroundings. Applications of this knowledge also bring about changes in society and the cultural order and may have a direct bearing the quality of life.

The impact that science can effect on the quality of life is well documented in the normal everyday events that surround our lives. Any parent of young children can attest to the advancement of pharmaceuticals and the role that they play in the health and well being of their offspring. As parents, we accept prescriptions for the care of childrens' illness and rarely hesitate to follow doctors directions about treating our children with medication. The development and government

approval of antibiotics and other medicinal drugs is a long and expensive journey. A pharmaceutical company can hardly afford to run up a large tab in research and development only to have the general population turn its collective back in fear and ignorance. The development of a biotechnically literate society will be crucial as the global population experiences the challenges of the future. Heading the list are new and more clever pathogens (HIV and Ebola viruses for example), the greater dependency on efficient agriculture to feed an expanding population, as well as, the protection of the biosphere as we continue to destroy unexplored environments and contaminate our habitat. "Nations and cultures are increasingly dependent on one another through international economic systems and shared environmental problems" (Science For All Americans, Project 2061, 1990 p. 102). A global society that is ignorant of the technological advances of biotechnology will behave with mob like mentality and stand in the path of potentially beneficial research. This fear is a reality and was documented during the mid 1980's in Brentwood, California. Gordon Meeks summarized:

> When Advanced Genetic Sciences Inc. wanted to test its new product, Frostban, on a strawberry patch, nearby residents in Brentwood, Calif., protested and took the company on in a court battle that lasted four years. Although the company maintained that the product was safe and would save growers expensive frost damage to fruit, residents charged that the genetically altered bacteria could harm the environment and that the company had not done adequate safety tests. When the court battle ended last spring [1987] and the company was given the go-ahead for its tests, someone uprooted most of the 2,400 experimental strawberry plants (Meeks 1988).

It would be nice to think that society has moved forward in its collective attitude since the mid 1980's, but a decade later the fear of biotechnology still exists and has been recently manifested in the form of a Wisconsin State Law passed in 1990 banning the use of recombinant BGH/BST in the dairy industry

of Wisconsin "because of its potential impacts on the wholesomeness of milk and on the sustainability of small dairy operations. The case of BGH/BST illustrates that the public will have a role in the decisions to develop and market a new biotechnology..." (Zinnen, 1993).

The relationship between science, technology and society, fostered by science teachers, has been well delineated by Dowling; et al. In the publication: A Guide to Curriculum Planning in Science, 1986, Dowling sites specific associations (table 4):

> **Attitudes and Beliefs**. Progress in science and technology is controlled by the attitudes and beliefs of society which are, in turn, influenced by the progress of science and technology.
>
> **Economics**. Science and technology influence economic growth which, in turn, affects the progress of science and technology.
>
> **Politics**. Science and technology influence political processes which, in turn, affect the progress of science and technology.
>
> **Societal Needs**. Developments in science and technology affect the condition of life, and, in turn, society's response to scientific and technological developments affects the direction of research and development.
>
> **Personal Needs**. Science and technology can affect individual lifestyles and the individual, in turn, responds to affect science and technology.

The role of the average secondary science teacher is becoming more demanding and increasing critical in the biotechnical era. As is evident by the state of Wisconsin Science Curriculum Planning Guide, curriculum planning is calling for a paradigm shift in science education from covering vast quantities of content to conceptual understanding and technological literacy. This call for a paradigm shift has been reiterated over the past decade in various publications, including the 1993 BENCHMARKS for Science Literacy, and the National Science Education Standards (NSES), published in 1996 by the

National Academy of Sciences:

> If students themselves participate in scientific investigations that
>
> progressively approximate good science, then the picture they come away with will likely be reasonably accurate. But that will require recasting of typical school laboratory work. (Benchmarks, 1993; p. 9) Overestimation of what students can learn at a given age results in student frustration, lack of confidence, and unproductive learning strategies, such as memorization without understanding. (Benchmarks, 1993; p. 327)
>
> In grades 9-12, students' understanding of biology will expand by incorporating more abstract knowledge...Teachers of science will have to make choices about what to teach that will most productively develop student understanding of the life sciences. All too often, the criteria for selection are not clear, resulting in an overemphasis on information and an underemphasis on conceptual understanding. (NSES, 1996; p. 181)

The problem of content driven curricula has been around for a very long time and apparently attempts to change the course of science education have met with great resistance. Consider comments from authors nearly one hundred years apart.

> A great difficulty in teaching Botany is to determine what are the most profitable topics for consideration. The trouble with much of the teaching is that it attempts to go too far, and the subjects have no vital connection to the pupil's life. (L.H. Bailey, 1903)
>
> Educators agree change is needed across the curriculum. Yet there is a rare revolution that meets no resistance, and this [change in science curriculum] is no exception. Professor Anderson [Michigan State's Institute for Research on Teaching] and colleagues, for example, have tested new approaches that emphasize depth, not breadth, with significant success. Yet teachers resist. Many don't see the point in "all the extra stuff between the facts," Professor Anderson said. They think the new material is "watered down". (K.C. Cole, 1990)

Plant biotechnology is nothing new. Plants have been utilized, manipulated and engineered for thousands of years by cultures all over the globe. The most widely recognized examples include the fermenting of

carbohydrates into alcohol by yeasts, the cultivating of rice into a high yield crop and the genetic selection and controlled pollination of corn to produce the large ears of sweet corn today from the small low yielding corns of yesteryear (Science For All Americans, Project 2061, 1990 p. 61). Plants remain in the forefront of biotechnology today, due to the "relevance of plant research to the immediate needs of humanity" "We're currently feeding half the world"(Thimann 1988, Biology N. Campbell p. 671, 673). Geneticists are using recombinant DNA technology to insert genes into plants to produce pest and disease resistant qualities into test crops. This research is geared toward the eventual use of these plants in high yield monoculture crops. Most of the land in the United States that can be used for agriculture is already being used. We must begin to look at ways to use other land not traditionally slated for agriculture, particularly arid environments (Thimann 1988). "Farmers may be growing crops year round that defy drought, make their own fertilizer and manufacture medicinal drugs, it sounds like science fiction but its not." (J. Dyson, 1992).

These activities are not progressing unnoticed by the "anti-biotechnology" watchdogs of our political society. This is both good and bad for the overall progress of biotechnology: good in the sense that it forces scientists to police themselves and the activities of their colleagues, but bad in the sense that it can greatly slow progress and drive up the cost of testing and implementation of technology that has the potential for bettering the world that we all share.

The onus is upon science educators to step up to the challenge of preparing our students to compete and survive in the twenty first century. This is not a role that will be taken on by the business or governmental sectors of our society. There are examples of such attempts, for example when NutriSweet (Aspartame) hit the market. Commercials were aired telling of how the product

was produced from milk and bananas to assure the public of the product's safety. This was a marginal effort, and was obviously driven by the bottom line 'how can we sell this product and make money?'. The only sure way to develop a technologically literate society will be through efforts of teachers, especially science teachers, to inform and foster an understanding and critical acceptance of the principles and practice of biotechnology.

DESCRIPTION OF ACTIVITIES AND OBJECTIVES

The first group of activities include the use of the following techniques:

A) Paper and column chromatography used to separate compounds of similar solubility or polarity. This technique is employed in biotechnology labs for the purposes of isolating a desired compound from a mix of related compounds.

B) Protein extraction and spectrophotometric analysis. The spectrophotometer is utilized as a detection device for determining the presence of a product or microbe density.

These activities also reinforce the techniques of graphic analysis, pipetting, use of indicator reactions, serial dilutions, solution and buffer preparation.

The second group of activities include:

A) Sterilization, aseptic technique, and media preparation required in all biotechnology labs that employ the use of microbes. Also required on large scale plant propagation operations such as potato farming (tissue culturing).

B) The effects of external factors on the growth of an organism. Organisms and microorganisms are often used as indicators of environmental quality and for studying the impact of mans activities on the biosphere.

These activities were taught over the course of two years to four classes of advanced biology students (two classes each year). They ranged in grade

level from sophomores to seniors and there was no requirement that students had previously taken high school biology, although about one third of the students had completed one year of introductory biology. The only common course requirement was that all students enrolled in the class must have successfully completed one year of high school chemistry. Group one students were exposed to all the of labs that the group two students were with the exception of the plant tissue culture lab. All classes were presented with the same course content. All classes were taught at a large comprehensive high school of 1600 students. The community population exceeds 55,000 people and there are two high schools in the district, both of similar size and population.

CENTRAL GOALS TO BE MET BY THE ACTIVITIES

The primary outcome of these activities is to improve the level of confidence and performance of students in the laboratory setting, and to develop an informed and positive attitude toward biotechnology and the role of plants in the biotechnical arena. While the introduction of new techniques and activities will be highlighted, the emphasis on problem solving and cooperative learning will be retained. Students who have completed the class will be given a two fold survey after leaving school for the year. The ordinate survey ask the student to respond with a number from one to five (negative to positive) to two series of questions. Participation in the survey is completely optional. The two tacks of the survey are a) student perception of their competence in the lab, and b) students perceptions of the role of biotechnology in our society. Through the completion of the activities presented in this document the following goals will be addressed:

Goal One: High school students can learn to use and apply the following laboratory skills and techniques that are employed in biotechnology:

A. Chromatography

B. Spectrophotometry

C. Pipetting/Micropipetting

D. Aseptic Technique

Goal Two: Students will be more likely to recognize plants as a biotechnological tool after the completion of the activities described within this document.

Goal Three: Students perspectives and attitudes toward biotechnology will be more positive after the completion of the activities described within this document.

SPECIFIC OBJECTIVES

1. To develop the skill of collecting and reporting data. The student will keep accurate records of all lab activities and present them to the instructor in an organized formal lab write up format. (See Goal one).

2. To develop the skill of solution preparation. The students will prepare all of their required solutions for all lab activities including the preparation of buffers required. (See Goal one).

3. To develop the skill of pipetting. During the lab activities the students will use pipets and micropipets to transfer and measurement of required solutions . (See Goal one).

4. To develop the skill of using chromatography. The student will separate water soluble ink and an extract of plant pigment using paper chromatography. The student will also learn the technique of using Reverse-Phase Column chromatography to separate an unknown mix of food dye. (See Goals one and two).

5. To develop the skill of using spectrophotometry. The students will use the Spec 20 to measure and plot the Absorbance of protein samples of varying

concentrations. They will also apply the use of the Spec 20 to identify the amount of protein extracted from a food sample, and analyze a pigment extract from plant tissue. (See Goals one and two).

6. To develop the skill of aseptic technique. The student will learn the use of a "pressure cooker" autoclave in the sterilization of materials. The student will demonstrate aseptic technique in the preparation of plant tissue for culture. (See Goals two and three).

7. To learn the procedures involved in tissue culturing and the effects of two hormones on the differential development of plant tissue. (See Goals two and three).

8. To learn about the effects of the environmental conditions on seed germination rates. The student will prepare and utilize a mini-growth chamber for a controlled experiment using Wisconsin Fast Plant seeds (*Brassica rapa*) . (See Goals two and three).

DESCRIPTION OF LITERATURE USED FOR THE PREPARATION OF ACTIVITIES

The text available to the student for use is Neil A. Campbell's: Biology, 1990 - Second Edition published by Benjamin/Cummings Publishing Company, INC. Descriptions and discussion of the tools of biotechnology are scattered throughout the text in places too numerous to list. Correlations to text material were made where appropriate to the class activity in question.

The High School Biology and Chemistry Teacher's Research Manual for the summer workshop in molecular biology at Michigan State University (Summer 1988 and 1989) was used extensively in the development of activities and lecture material that relate directly to this document.

Reference texts include: Biochemistry, Stryer, 1987; Biochemistry, Champ and Harvey, 1987; Carolina Plant Tissue Culture Media Formulation

Booklet, 1986; Wisconsin Fast Plant Manual, 1989 and 1990; Eucarpia Cruciferae News, Newsletter #7; Pierce Chemical BCA Reagent Manual; J.T.Baker Mini-Prep Column Chromatography Literature.

The first set of activities, objective numbers 1, 2, and 3, acquaint students with the reporting of data. The materials and techniques of the laboratory such as, basic chemistry, solution preparation and pipetting are skills that should be within the capabilities of the science teacher. Various laboratories from the Michigan State University/National Science Foundation Summer Workshop Manual were also adapted to perform the activities described in this document, objective numbers 1, 5, 6, and 7. They include: Preparation of a Formal Lab Write Up, pp. 21-22, revised in 1990 and 1995 see Appendix A; Tobacco Leaf Tissue Culture, Lecture by Dr.H. Hagerman June 22,1988, revised in 1991 and 1995 see Appendix E; Preparation of pH Specific Buffers, pp. 25-30, revised 1990 and 1995 see Appendix B; Preparation of a Standard Curve, pp. 90-91.

Textbooks often mention chromatography, objective number 4. The 1989 research manual provides instructions, reference tables, and an example lab on pages 72-75 for the separation of dyes in kool aid. The lab that is used in my classroom as an introduction to chromatography was developed by Richard Feezel during the summer of 1989 as a result of participation in the molecular biology individual research group. A revision of this activity is located in Appendix C of this document.

Spectrophotometry, and the analysis of protein, objective number 5, is also detailed in the MSU/NSF Summer Workshop Research Manual 1989, pages 30-37, and pages 60-64. The activity detailed in Appendix D is a 1995 revision of a lab developed in 1988 by Richard Feezel, et.al.

Aseptic technique, objective number 6, is employed during the course of the year but must be revisited for the purposes of tissue culturing. This is

discussed on page 47 of the MSU/NSF Summer Workshop Research Manual. Pages 47-48 are also used in the preparation of plant tissue for culturing, objective 7, revised in 1991 and 1995, see Appendix E.

The publication DNA Science, pages 199-214, is utilized in the preparation of student work with micropipets and aseptic technique, objective numbers 3 and 6.

In order to perform the activities involved in objective number 8, the students must be familiar with Wisconsin Fast Plants. The Wisconsin Fast Plant Program, University of Wisconsin-Madison, Department of Plant Pathology has produced a manual entitled Wisconsin Fast Plants Growing Instructions that explains the procedures and materials required to address objective number 8. This a forty page document that is necessary for an instructor unfamiliar with Wisconsin Fast Plants. Data pertaining to the growth of Wisconsin Fast Plants is detailed in Appendix I.

NEW TEACHING TECHNIQUES

The greatest change in teaching style has come from converting the laboratory format of my classes from the traditional 'do this and expect to see that' to an open ended inquiry based lab format. The students are able to generate ideas pertaining to concepts or techniques discussed in class and turn them into labs that they perform. Since I still have a major role in how the labs play out, this might be better called guided inquiry. My role as the instructor is to plant the seeds of curiosity, demonstrate the tools available and then assist the students in performing activities commensurate with the objectives of the class.

I have also changed the percentage of time that students are in their seats either taking notes or working on questions and problems pertaining to the class objectives. Students now spend more time in cooperative learning groups mastering concepts or performing lab activities that they help design.

The student cooperative groups varied every ten weeks to ensure that the students did not also always fall into the same lab situation role. By this I mean that 'students X', due to the nature of those students in the lab group, would not always end up doing the recording of data, or preparing of solutions. This forces change in the lab routine and students assume different lab roles throughout the year. By maintaining lab groups for a period of ten weeks, it also motivated the lab groups to find ways to make their lab groups work well and be successful, as the success of the lab depended on their collective interdependent effort.

My teaching style has changed for both my introductory level classes and my advanced level classes since participation in the Michigan State University/National Science Foundation Summer Workshops in Molecular Biology, Research and Environmental Biology. Although it is not documented in this thesis, I know that my students are getting more from biology and from myself as teacher due the changes described above.

CHAPTER TWO

DESCRIPTION OF LAB ACTIVITIES AND RATIONALE

Each of the following activities and labs are new to my classroom and would not have been developed to their current successful format without my participation in the Molecular Biology Summer Institute offered through Michigan State University, Division of Science and Mathematics Education, and the National Science Foundation. The activities described within this document address the curricular goals of teaching Science-Technology-Society and Biotechnology as is suggested by the State Of Wisconsin Department of Public Instruction. The activities described in this section are all the direct result of my participation in the summer institute. The labs were developed and revised from Summer Institute and participant generated lab activities

FORMAL LAB WRITE UP

Before most students enter my advance placement biology class they have little or no experience in writing their own lab reports. The usual method from teachers that I have had contact with has been the typical 'cookbook' style lab. The students receive lab sheets and simply follow the instructions. There is little discovery or science going on with this format. This has been my experience in three states and seven different school buildings where I have been a teacher for the past fifteen years. In order to change my own teaching style I now use the following format.

The students will be introduced to the practice of preparing a formal lab write up during their first lab work on preparing solutions. It is important to allow the students to get their feet wet in the lab without the spectre of a formal report

staring them in the face before they even acclimate to the lab and their lab partners. Once the first set of solutions has been prepared the students are more comfortable and are able to produce a product that is very satisfactory for the first lab experience. The first formal lab write up begins with the preparation of a buffer and concludes with serial dilution activity. Here the students must title their lab and determine their objectives and procedure that they will follow considering background and research that they have done or been exposed to in class. They report their data, calculations, and error analysis. The instructor will provide questions to guide their conclusion.

From this lab on, all activities will follow the same formal lab write up format unless otherwise indicated by the instructor. The format recommended as a base lab format mirrors the format described in the introductory pages of the summer institute manual, see Appendix A.

SOLUTION AND BUFFER PREPARATION

The students prepare solutions of varying concentrations as described by the instructor. The solutions will include potassium phosphate (monobasic, dibasic and tribasic), and TRIS and Trizma HCl. The phosphate solutions will then be used to make buffers of pK values equal to 6.9 and 2.8. The Tris/Trizma HCl will be needed to prepare buffers with a pK value of 8.2. The students must have their calculations checked prior to making the solutions and storing them for future use.

The students will then be instructed on the function and preparation of a buffer. A derivation of the Henderson-Hasslebach equation will serve as the basis for proofing the process of preparing a buffer. Students will be provided with a conversion chart for determining the acid -base ratio to be used in the preparation of a pH specific buffer. Student calculations and percentiles of each chemical will be checked by the instructor before continuing.

Once buffers are prepared, students will check their buffer's pH for accuracy and hypothesize how to adjust the pH of their solutions without changing the concentration of their product. The final pH value of their solutions needs to be within a of five percent error range of the expected values assigned.

The third part to this activity is to investigate the effect of dilution on the ability of a buffer to maintain its pH. This is accomplished through a serial dilution of the buffer with distilled water. The students are asked to prepare solutions of decreasing concentrations without using all of their stock buffer up in the process. The students hypothesize how to do this within their own lab groups and present their hypotheses to the instructor before proceeding. This allows the instructor an opportunity to guide the individual groups into discovering the process of serial dilution and lend only enough assistance to each group to reach the same conclusion on their own. The process should be summed up by the instructor with the whole class so that students have the opportunity to ask questions and be confident in their skills, as they will be required in other lab work throughout the remainder of the year.

Another objective addressed by this activity is the learning of correct procedures for pipetting and micropipetting. To achieve the desired acid/base ratios or dilutions students will need to pipet solutions using both standard pipets and micropipets.

Although students have all had at least one year of high school chemistry, they have very little confidence in their ability to prepare and use solutions. By the end of these three activities the students exhibit increased confidence and expertise in the lab setting.

These activities were developed, tested and revised over the course of several years by R.D. Eicher, and are a direct result of participation in activities

of the MSU/NSF Summer Institute.

CHROMATOGRAPHY

During the second major unit of the year the students use chromatography as a method for the separation of organic compounds from a solution. Class discussions focus on the use of this technique in biotechnology research labs to isolate compounds for further study or use with an organism. The fact that students might be able to employ this tool is very exciting to them.

Students are first asked to do a cookbook lab activity that will familiarize them with the process of both paper and column chromatography.

The first activity involves using a plant of their choice to extract chlorophyll and utilize paper chromatography to separate the pigments from the extract. The set up is demonstrated by the instructor using water soluble ink, such as that found in a Vis-a-Vis marker. This allows the students to gain insight about the procedure without giving away the results expected from their chromatographic separation of plant pigments. The students choose their own samples of plant tissue to study. The class results show the varying pigmentation content of different plants. Each group will report to the class at the conclusion of the activity.

The second activity involves the separation of dyes used in the coloring of kool aid. Each group is given a protocol for the separation of grape drink mix dyes. They can readily determine that the color of grape drink mix is purple and the dyes that can be separated will be red and blue. In order to perform this separation the students will need to be taught to use a mini prep reverse phase chromatography column. The column, produced by J.T. Baker Company Inc.,is packed with and octadecal/silica matrix that can be adjusted in polarity by eluting different concentrations or types of solvent through the column. By adjusting the polarity of the effluent students are able to get the dye from the

kool aid to adhere to column. Once the dye is on the column the students are able to separate the red and blue dyes by eluting solvents of varying polarity through the column. The students must prepare their own solvent solutions of varying polarity. Students learn the technique and process of reverse phase column chromatography, and the skills mastered in the first set of labs on solutions are reinforced and improved.

The first two activities described here are precursors to the main chromatography activity. Each group brings a food sample that contains food dyes that can be extracted and isolated. This activity requires a formal lab write up, and it is likely the first time the students are required to perform a real problem solving, inquiry based lab. It tests their ability to work cooperatively as a group, map out a strategy for solving a problem, implement that strategy and report on the results of their work. Students are encouraged not to bring a sample in liquid form so that they are forced to extract the dye from sample first and then succeed in loading it onto their column. This lab has been very successful. It boosts student confidence in the lab and changes their approach to labs from "we have to do this?" to "we can do this!". This series of labs requires some time commitment, but the payoff in student confidence and competence is well worth the three to four days of lab time.

This series of activities are based on research work done by Richard Feezel, et.al. during the MSU/NSF Summer Institute, 1989, and later developed into their current form by R.D. Eicher. This activity is detailed in Appendix C.

SPECTROPHOTOMETRY

Like chromatography, spectrophotometry is a technique employed by many biotechnology industries to indicate the presence and concentration of chemical products. It is also used to determine the population growth rates of bacterial suspensions. Spectrophotometry is an important skill to be developed

in an advanced biology class. Spectrophotometry is a technique that will be utilized through the course of the year and is introduced early on in the study of organic compounds. Students are introduced to spectrophotometry as a detection technique by being shown two solutions of similar color, one being food coloring and the other being a solution of Eriochrome dye. Both solutions appear blue/black, however only one exhibits a reading of Absorbance on the spectrophotometer at 528nm! It can not be expected that students will be able to "discover" how to run and utilize a spectrophotometer, so they must be instructed on the proper procedures and protocol for operation. Once the students have been thoroughly instructed, they are directed to prepare protein samples of varying concentrations and asked to determine the percent transmittance, convert to Absorbance and graph their results. The stage has now been set for some scientific work. The students are presented with a sample of unknown concentration of protein and asked to determine a method for determining the value of the unknown. Once again the students work in groups and must prepare a strategy. Proposed procedures must be cleared by the instructor before the groups can continue. This permits the instructor to move the students in a reasonable direction.

Students are asked to bring in a sample of pet food with an identified protein content on the label. Students are to determine the concentrations of the unknown proteins by two methods: 1) Plot the unknown on a standard curve, prepared earlier in the lab using serum albumin; 2) By calculation, using the Beer-Lambert equation.

Once the students have determined the concentration of the unknown, they can check with the instructor for the accuracy of their results. Students then can continue with the extraction of protein from their pet food sample and compare their results to the value stated on the bag by percent error analysis.

These activities allow the student to perform quantitative analysis while learning how to employ the use of a spectrophotometer. Students draw conclusions comparing their results, by calculation and graphic analysis, to known values from the product information. The student results are usually quite humbling. They come away from the lab with respect for the analysis of food samples for their organic compound content.

These activities are based on the protein lab sessions from the MSU/NSF Summer Institute, and the activity involving a pet food sample analysis is a variation of an institute participant lab activity by Richard Feezel, et. al., 1988. See Appendix D.

PLANT TISSUE CULTURING

Prior to this series of laboratories, the students have studied cells, cellular respiration, photosynthesis, cell reproduction and genetics. They have some exposure to concepts surrounding the structure and function of plants, their cells and energy systems. They have been assigned readings that highlight plant evolution, classification and life cycles of the major plant groups.

The plant tissue culture activity is one that is very labor intensive and mirrors the propagation process used by potato farmers in the Midwest. The students will require detailed instruction along the way. One of the first requirements is that students need to understand the terminology, equipment and materials that will be utilized for the culturing of plant tissue. In lab groups, the students are asked to research using their textbook: plant tissue culture, culture media, aseptic, hormone regulation, auxin, cytokinin and autoclave. This exercise should initially familiarize the students with terminology and background so they will be more knowledgeable as instructions are given.

Each lab group is assigned a different aspect of the lab to set up. One group is to prepare the culture media for the entire class. The use of pre-

prepared hormone and salt powder is utilized and the instructions are provided. Another major task is to prepare the autoclave (pressure cooker) and all of the hardware to be sterilized. While some group members are working on common tasks for the classroom materials, other members of the individual groups are gathering, preparing and labeling the materials that will be used for their own lab group. This will require one class period, and the instructor will need to run the autoclave when all the materials are prepared. Once all the materials are prepared, the members of the individual groups have the opportunity to compare notes and update their lab reports, as there are often additions or modifications to the procedure as the lab progresses. The instructor will likely need to do successive autoclave runs to ensure that the activity's time line remains on track.

The students proceed with aseptic technique to prepare their samples of plant tissue. Chrysanthemum leaves were used for tissue culturing in this activity. Students are reminded to be cautious in the lab due to using open containers of ethanol (for sterilizing hardware) while alcohol or Bunsen burners are running at the same station.

Once the prepared tissue cultures are placed under the light bank and the lab is cleaned, the lab groups prepare data charts to record daily observations of their cultures.

This activity will generally run for around four weeks, provided that there are uncontaminated culture tubes remaining. This needs to be ongoing in status to allow for the cells to differentiate and students to be aware that they are responsible for maintaining data collection on a daily basis. The biggest pitfall is contamination of the tubes. The students may need to prepare some replacement tubes so that have so successful cultures to observe.

High school students can be frustrated with this experience because the

success rate is often quite low. However it is an excellent learning activity in that students can garner a new appreciation for the measures that need to be taken for the success of a lab requiring aseptic technique and conditions.

The logistics and fundamentals of this activity were adapted for classroom use based on the Tobacco Plant Tissue Culture Activity performed in the MSU/NSF Summer Institute, 1988. See Appendix E.

SEED GERMINATION AND ENVIRONMENTAL IMPACT

Wisconsin Fast Plants, or Rapid Cycling Brassica's (RCB's) were used so students could investigate the effects of an environmental factor that is of interest to them. Factors vary from radiation doses and wavelength of light, to fertilizer concentration and application of pesticides, plus many others.

The speed at which these plants germinate and develop satisfies the students needs for something to happen relatively quickly, an expectation that they don't normally have with plants. This also facilitates data collection in a short amount of time compared to other labs that utilize plant tissue. Control plants normally exhibit signs of germination overnight, and the experimental plants will germinate a short time later or sometimes even sooner than the controls depending, on the students experimental design. The rapidity of the set up of this activity also allows for several experimental runs when problems arise. This allows students to analyze and rework their hypotheses when things do not proceed as they had planned. This is a powerful teaching tool for students as they run, analyze and rerun an experiment of their own design. Another positive aspect of utilizing RCB's, is that they need relatively little attention to grow and yield experimental results, and they do not elicit a negative "attitude" from the animal rights sympathizers in the classroom!

The core piece of equipment for this activity is students own mini-growth chamber designed by R.D. Eicher in 1989 and revised in 1995 specifically for

the purposes of this activity. The chamber is very simple and consists of nothing more than a small water tight container, such as a yogurt or margarine tub, a microcentrifuge tube and piece of string (see Figures 4 and 5, p. 108, Appendix L). The mini chamber's design is modeled after the self wicking/watering system designed for the RCB,s by Dr. Paul Williams, et al. at Wisconsin Fast Plants-University of Wisconsin-Madison, and marketed exclusively by Carolina Science and Math. I have found the mini chambers to be good for performing labs of this nature, and they are logistically easier to work with in the lab than the large containers supplied by Carolina Science and Math. Students also supply and make their own chambers lending a student ownership quality to the experimental set up.

The class is introduced to RCB's in discussion format and the problems that may arise in doing their labs are discussed. The instructor then works into the discussion the advantages of the mini growth chambers and describes the design and the materials needed to run the activity. The student lab groups can now proceed in designing their experiment without the worry of the how's, what's, and why's of the physical set up.

Each lab group is given the task of determining an environmental factor that they perceive to be a potential problem for the germination of a plant seed. Once this has been selected, they are to determine how they will test this factor within the parameters of the experimental set up. All designs must be cleared by the instructor for relevancy and logistical practicality before the students proceed. Students usually propose some interesting factors that they wish to test. Examples include; proximal voltage, varied concentration of Miracle-Gro, acid rain simulation, and radiation exposure. Each group is to prepare both an experimental and a control mini growth chamber with multiple sites for several planting positions, prepare any solutions or additives that their protocol calls for

and run their lab. They must also determine what values they will measure in their experimental and control groups. Individual students are to keep their own records for their formal write ups, as well as prepare conclusion statements at the end of their lab.

All plants, unless directed by the experimental design, are grown under standard forty watt fluorescent lighting twenty four hours per day, as per instructions for growth of RCB's. Students need to be careful about keeping the water in their mini growth chambers at an appropriate level so their RCB seeds or seedlings do not dry out, or become water soaked and disrupt their experiment. Ideally, the students should be able to grow their plants until they can pollinate and harvest seeds (see Figure 3, p. 101, Appendix I).

Harvested seeds could then be used to grow a second generation of plants to gather data concerning the effects of their experimental factor on the germination of these progeny plants. My experience using RCB's in lab activities is that the second generation plants do not grow very well. It should then be expected that seeds grown under stressful conditions also exhibit difficulties in growth. This, along with the quickness that results can be obtained, are the primary logistical reasons why germination of seeds (and not their progeny) have been selected as a method for using and studying fast plants. Logistics aside, this activity provides students with an opportunity to: 1) investigate the conditions under which plants must live and grow; 2) observe the growth of plants; 3, identify structures as they present themselves in the lab; and 4) care for an organism for the purposes of their own research.

This activity was developed directly as result of participation in the MSU/NSF Summer Research Institute, 1989 by R.D. Eicher. This activity is detailed in Appendix F.

PLANT PIGMENTS: CHROMATOGRAPHY AND SPECTRAL ANALYSIS

The students have worked with both of these techniques earlier in the year and now must call upon their experience, and their lab notebooks, to guide them through this activity. The objective of this activity is for students to recognize that there is more than one protein pigment involved in the collection of light energy by a plant. They can show this by using techniques that they have developed to extract, separate and analyze a pigment sample from a leaf.

The choice of plant material in this activity does not matter a great deal so long as the tissue selected is fresh. Students perform an extraction of the plant pigment using a mortar and pestle grinding the tissue with a small amount of chromatography solvent. Using the extract students run a paper chromatograph of the extract and let it dry for analysis the next day.

With the successful completion of the pigment separation the students can isolate their bands of pigments by merely cutting the paper strips apart along the lines that define each band.

The lab groups must now determine the best method for removing the pigment from the paper for the purposes of analysis. This is achieved via class discussion so that all the groups are working under the same set of directions. Discussion is directed so that students reason out a practical method to remove the pigment from the paper: immersing it in a cuvet containing three milliliters of chromatography solvent. Students are to determine the Absorbance spectrum for each sample using range of 350 nanometers to 700 nanometers, recording data every twenty nanometers.

Students graphically analyze their data to determine if they did separate pigments and speculate as to the identity of those pigments based on: 1) their graph of Absorbance as a function wavelength; and 2) the color from their paper

chromatograph. Finally each lab group reports its findings to the class so comparisons can be made between the different plants selected by various groups. Comparisons may include different numbers of bands, differing colors of bands and similarities between each of the groups' results.

This activity was developed by R.D. Eicher and is, in part, based on research labs developed by Richard Feezel during the MSU/NSF Summer Research Institute, 1989. See Appendix G.

IN VITRO POLLEN TUBE GERMINATION

The use of Wisconsin Fast Plants, Rapid Cycling Brassicas (RCB'S), can be quite diverse in the classroom. The students are aware by now how plants carry out sexual reproduction by transferring pollen from the antheridia of one plant to the stigma of another, which in turn initiates the growth of a pollen tube that will deliver the nuclei to the seed for fertilization. The goal of this activity is to show students that the chemical environment surrounding the pollen grain can be manipulated to induce *in vitro* growth of the pollen tube that can be observed using a standard light microscope. In order for this activity to be performed the students must have been successful at growing RCB's and producing flowers with viable seeds and pollen.

Students will be working in lab groups to facilitate the successful preparation of their sugar/salt solution, and to ensure that the activity is running by the end of the class period. Once the solution is prepared, several drops are placed into the well of a standard depression slide. Students are to collect pollen samples from the antheridia and transfer them to the microscope slide well containing the solution, and then be patient. Students should observe the pollen grains initially and then once every fifteen minutes until germination begins. The initiation of pollen tube growth may take as much sixty minutes, and given the time allotted for a typical high school class period, students will

need to arrange to return to the classroom throughout the course of the day to make observations and record their results. For an introductory biology class, the instructor might choose to set up this as a demonstration ahead of time. This allows for observation of pollen tube growth within one class period.

Due to the time restraints this activity does not lend itself to formal quantitative analysis and students will need to treat this lab as purely qualitative. This does not diminish the impact of being able to manipulate the growth of a pollen tube by *in vitro* technique, and the students find the lab interesting and worthwhile.

This laboratory exercise can be coupled with any activity where RCB's are being grown, or it can be used as a stand alone lab where RCB flowers are provided by the instructor for use by students. This activity lends itself well to teaching the anatomy of a flower, especially if each student can have their own flower to investigate and collect pollen from. The activity can also be varied for the level of student who is performing the lab. For an introductory biology student with little or no chemistry background, the sugar/salt solution would need to be provided by the instructor. For the advanced student, they can be assigned the preparation of the sugar/salt solution, with the success of their lab depending on the abilities of their lab group to accurately prepare a solution. Either method will result in a good lab experience for the students.

This activity was developed by R.D. Eicher, during the MSU/NSF Summer Research Institute 1989, and based on material described by Hodgkin, Marr and Wiseman (1982). See Appendix H.

CHAPTER THREE

EVALUATION AND ASSESSMENT

The introduction of each activity/unit began with either a pretest or an informal pre-unit discussion to assess the students general knowledge. At the end of each unit there is an exam that includes items pertaining to the activities of this document. Refer to Appendix J for sample items. Test questions include both objective and performance items that required the student to solve a problem. Students were also asked short answer/essay questions regarding some of the technical skills which were used in a given activity.

Authentic assessment of students work was measured by the quality of the students lab reports of the activities performed. See Appendix A for a sample grading form for lab reports.

The final form of assessment that was utilized for the purposes of this thesis was an exit interview/survey that was mailed out to students who completed the course and activities during the 1994-1995 school year (group one students), and the 1995-1996 school year (group two students). This survey, parts 1 and 2, was the most important evaluative measure for me in terms of this thesis. At the conclusion of this two year period, 1994-1996, I sent fifty-eight surveys out to students who had completed my Advanced Placement Biology class and asked those students to complete the survey and return them to me. The surveys did not ask for the students to indicate their identity, so as to maintain their anonymity. Of the fifty-eight surveys mailed I received forty-five back, a respectable return of seventy-eight percent. I did not want the students to feel they could not be anything but completely honest and anonymous in their

responses to ensure the validity of their responses on the survey, in short, I believe that I received honest feedback from my students.

The items on the survey addressed the impressions of the students confidence level in performing lab activities. The survey further explored the students attitudes toward biotechnology and their future in science education.

The results of the survey are divided into responses from group one and group two. Responses are compared by: 1) year to year results; and 2) the retention of impressions and attitudes by students who have been out of the class for a period of year. The difference between the group one students (1994-1995) and the group two students (1995-1996) is that the group one students did not perform the Plant Tissue Culture or the Plant Pigment Chromatograph and Spectral Analysis activities. The concepts of tissue culturing, pigmentation analysis were covered in lecture/discussion format for group one students. The survey results were tallied and the means and standard deviations for each item are reported in Tables 1-3 and discussed in this section. The change in the mean values (Delta Value) are reported for each item on the survey, as well as the overall mean delta values for each portion of the two parts of the survey. The analysis of these results and the implications they suggest pertaining to the goals of this document are found in the Summation of Data section in the conclusion.

SURVEY PART 1: DESCRIPTION

Survey Part 1 asks the students to rate their confidence level in a variety of performance skills that were addressed directly by the activities outlined by the document objectives. The students were asked to rate 15 items on a scale of one to five based on their confidence level in performing the tasks identified for both before and after the completion of the course. A rating of one indicates zero confidence in the ability to address the task in question. A rating value of

five indicates an excellent level of confidence in the ability to address the task in question. A discussion of the comparative analysis for the first survey section can be found immediately following the tables one and two showing the statistical summary of survey part 1. See Appendix K for the text of Survey Part 1.

Table 1 - Results of Survey Number One 1994-1995

Item Number	Mean Before (SD)	Mean After (SD)	Delta Value
1	1.06 (0.250)	3.94 (0.929)	2.88
2	1.56 (0.727)	4.19 (1.223)	2.63
3	1.63 (0.719)	4.44 (0.727)	2.81
4	2.63 (0.885)	4.69 (0.479)	2.06
5	1.75 (0.683)	3.88 (1.148)	2.13
6	1.44 (0.512)	3.31 (1.302)	1.87
7	1.06 (0.250)	1.69 (0.873)	0.63
8	2.06 (0.680)	4.56 (0.629)	2.50
9	1.81 (1.286)	2.88 (1.310)	1.07
10	1.50 (0.632)	2.94 (1.182)	1.44
11	1.44 (0.629)	3.63 (1.310)	2.19
12	1.69 (0.873)	4.0 (0.817)	2.62
13	1.25 (0.447)	3.0 (1.155)	1.75
14	1.19 (0.403)	3.13 (1.147)	1.94
15	1.25 (0.447)	2.56 (1.315)	1.31

Mean response value before : 1.55, standard deviation : 0.422
Mean response value after : 3.52, standard deviation : 0.834
Mean delta value (response change from before to after) : 1.99, standard deviation : 0.622
n = 45

Table 2 - Results of Survey Number One 1995-1996

Item Number	Before (SD)	After (SD)	Delta Value
1	1.38 (0.637)	4.23 (0.863)	2.85
2	1.77 (0.992)	4.35 (0.745)	2.58
3	1.81 (0.801)	4.54 (0.647)	2.73
4	2.85 (1.047)	4.62 (0.571)	1.77
5	2.08 (0.935)	3.96 (0.999)	1.88
6	1.62 (0.084)	3.69 (0.884)	2.07

Table 2 (cont'd)

Item Number	Before (SD)	After (SD)	Delta Value
7	1.38 (0.697)	3.77 (1.395)	2.39
8	1.77 (0.710)	4.42 (0.643)	2.65
9	1.58 (0.758)	3.65 (0.797)	2.07
10	1.62 (0.752)	4.15 (0.613)	2.53
11	1.65 (0.745)	4.42 (0.643)	2.77
12	1.73 (0.962)	4.15 (0.675)	2.42
13	1.73 (0.919)	4.69 (0.549)	2.96
14	1.27 (0.533)	4.11 (0.711)	2.84
15	1.31 (0.679)	3.96 (0.871)	2.65

Mean response value before : 1.7, standard deviation : 0.384
Mean response value after : 4.18, standard deviation : 0.33
Mean delta value (response change from before to after) : 2.48, standard deviation : 0.371
n = 45

SUMMATION OF RESULTS FOR SURVEY 1

Item numbers 1, 2, and 15 deal directly with the use of the spectrophotometer, objective number 5. Group one students average mean score these items 1.29 before completion of the activities in AP Biology and the average mean score for the same items after completion of the course was 3.56. The average delta value for these three items was 2.27. In comparison, the group two students average mean score for these three categories were: before,1.15, and after,4.18, with an average delta value of 2.69. The average delta value, or change in mean confidence, for these items is much lower in group one than in group two group one students because the group one students were only exposed to the procedures directly related to item number fifteen in discussion and text format, whereas, group two students performed the activity in lab. Referring to Table one, the results for item fifteen show a delta value of 1.31 for the group one students compared to a delta value of 2.65 for the group two students. It is clear in both groups, however, that the students

ability to collect and use information with a spectrophotometer was far greater than before completion of the course. Nearly all of the students (96%) surveyed in both student groups indicated that they had zero to little confidence in their ability to utilize a spectrophotometer before taking AP Biology. In comparison, 96% of the students surveyed indicated having at least fair confidence in their abilities in using the spectrophotometer, and most (80%) indicating good to excellent confidence levels after the completion if the course. Only 4% of the students surveyed indicated that they still had little confidence in using the spectrophotometer.

I was surprised by the results of item number three, objective number 3, micropipetting. In my past experience, teaching AP Biology in another school district, students had no prior exposure to using a micropipet. The student surveys revealed that some students (18%) must have had some previous exposure to using a micropipet as they scored item number three with fair confidence before the completion of the class. The most likely explanation for this is that some students are enrolled in the school district's talented and gifted program, and participated in an accelerated eighth grade science class that performed some activities in biotechnology. These same students scored their post AP Biology score for item three 71% excellent confidence level and 29% good confidence level in the use of a micropipet. The data show that all students gained confidence in their ability to perform exercises with a micropipet. Group one students mean score for item number three before the completion activities was 1.63, and after completion was 4.44 indicating that the group as whole reported between good and excellent confidence levels. The results for the group two students were nearly identical. Before mean score was 1.81 and after completion of activities mean score was 4.54.

Item numbers 4, 5, 6, and 12 assess the ability of the students to perform

some basic chemistry required for the completion of activities described within this document, as well as, working effectively in any advanced biology lab. These items address objective numbers 2 and 3, solutions, buffers and pipetting.

Item number 4 asks students to assess their confidence in their ability to accurately transfer fluids, specifically with a standard pipet. Students of both groups one and two report little to fair confidence prior to completion of the activities, group one reported a mean score of 2.63 (0.885), and group two reported a mean score of 2.85 (1.047). This was expected because students are required to successfully complete at least one year of high school chemistry as a prerequisite to enrolling in AP Biology. Both student groups report near equal confidence in this skill after the completion of the activities, group one reporting a mean score of 4.69 (0.479) and group two reporting a mean score of 4.62 (0.571).

Item number 5 on the survey deals more specifically with the preparation of solutions, objective number 2 (solutions and buffers). Both student groups report little to zero confidence in their ability to perform a task that should have been mastered during their year of chemistry. Group one students report a mean score of 1.75 (0.383) and group two a mean score of 2.08 (0.935). Students from both group report good confidence levels in this skill after completion of the course activities. Group one reporting a mean score of 3.88 (1.148) and group two a mean score of 3.96 (0.999).

Survey item number 6 asks the students about using serial dilution, a common skill used in biology labs where very small dilutions are often required. Both student groups report fair confidence levels of this skill upon completion of the activities, group one 1.44 (0.512) prior and 3.31 (1.302) after, and group two 1.62 (0.804) prior and 3.69 (0.884) after.

Item number 12 assessed understanding the purpose and preparation of buffers. It was not expected that students would have any prior experience with buffer systems before taking the course, although the students should have had some exposure to pH in their introductory chemistry class. Students in group one reported a pre unit mean score of 1.69 (0.873), and those in group two reported a mean score of 1.73 (0.962), showing zero to little confidence in making their own buffers. Their post activities mean score reflect a significant increase in their confidence levels to 4.0 (0.817) for group one and 4.15 (0.675) for group two.

Overall for the four items addressing objectives 2 and 3, the students reported an increase in their confidence to perform some basic, but very important and practical, chemistry skills in the lab. The average mean for all students in the survey for the four items before was 1.97 (0.838) and after the activities was 4.04 (0.859). The average increase for the composite of these items was 2.10.

Tissue culturing, objective number 7, and aseptic technique, objective numbers 6, were part of the curricula for both of the student groups. However, the activities were performed by members of the student group two, but only presented in lecture/discussion format to the students in group one. The difference in the understanding of this procedure is clearly shown by the data in survey items 7, 9, and 10.

Item 7 asks the student to assess their knowledge of an autoclave. Both group one and group two students show zero to little confidence prior to completion of the activities. Group one reports a mean score of 1.06 (0.250) and group two 1.38 (0.697). When asked to assess their confidence levels after activities, group one still reported little confidence, 1.69 (0.873), where as group students reported fair to good confidence. The mean score reported was 3.77

(1.395). The value for group two is lower than I would normally anticipate for an objective such this. However, due to the time restraints of running a lab activity in 53 minute blocks of time and the cycling time of the autoclave, most of the autoclave work was performed by the instructor.

Survey item 9 dealt with aseptic technique, objective number 6. Aseptic technique was employed during both years in either microbiology or in activities involving tissue culturing. Both students groups surveyed reported zero to little confidence before the completion of AP Biology, with group one reporting a mean score of 1.81 (1.182), and group two reporting a mean score of 1.58 (0.758). Their post completion score of 2.88 (1.310) showed little to fair confidence for group one in using aseptic technique in the lab. Responses of group two students were expected to be slightly higher in this category due to the time proximity of performing the activities involving aseptic technique to the completion of the survey. These students also had the opportunity to repeat their lab protocols for aseptic tissue culturing which would reinforce this objective rendering more positive results. Their mean score after working with these techniques was 3.65 (0.797) placing their mean confidence level between fair and good. It is also possible that their bouts with contamination had an impact on how they scored this item, making it the overall lowest scored item in the post activity column for the group two students.

Survey item number 10 was specific to tissue culturing, objective number 7. Tissue culture was performed by group two students and only discussed as a procedure with group one students. Both student groups report zero to little confidence initially, and improve their confidence levels to fair for group one, mean score reported 2.94 (1.182), and good for group two, reporting a mean score of 4.15 (0.613). It is apparent that there is a significant difference between the level of confidence in the ability to perform a procedure when the exposure

to the procedure is only presented not performed. This is evident in the mean fair confidence level in students who were exposed to the concept of tissue culturing through a discussion format only, compared to the good to excellent level of confidence expressed by the group two students.

The students that were surveyed for the purposes of this document all have completed at least ten years of schooling , with at least four years having classes that were specifically science classes. Yet, the ability to design an experiment really has not been developed during these years of schooling. Item number 8 on the student survey asks the student to assess their confidence level in producing a scientifically acceptable lab report at the high school level, objective number 1. Both student groups were required to prepare formal lab reports.

Students in both groups report little confidence in their ability to prepare a formal lab write up. Group one reported a mean score of 2.06 (0.680) and group two a mean score of 1.77 (0.710). After the completion of activities in AP Biology both student groups show a significant increase in their confidence levels reporting between good and excellent confidence. Group one's mean score was 4.56 (0.629) and group two reported a mean score of 4.42 (0.643).

Group two students had a larger block of time (four days) to work with the Wisconsin Fast Plants than did students in group one, although both groups performed the same activity. Item number 11 on the student survey asked the students about their confidence in preparing a mini growth chamber for the purposes of investigating seed germination in Rapid Cycling Brassicas (RCB's), objective number 8. Both student groups report zero to little confidence in this ability prior to the activities with RCB's. Group one's mean score was 1.44 (0.629) and group two had a mean score of 1.65 (0.745). Upon completion of the activities described both groups reported expected increases in their

confidence levels, group one reported an increase to a mean score of 3.63 (1.310) and group two students reported an expectedly higher mean confidence level increase to 4.42 (0.643) due to the additional time spent working with the RCB's.

The remaining two items from the survey, numbers 13 and 14, deal with application of chromatography, objective number 4. Both of the student groups had nearly zero confidence levels before participation in the activities described in this document. Group one students reported a mean score of 1.25 (0.447) and 1.19 (0.403) for confidence in the use of paper and column chromatography respectively. Group two students reported mean scores of 1.73 (0.919) and 1.27 (0.533) for the same items. Group two students performed all of the activities involving chromatography described in this document, where as group one students had a more limited exposure to chromatography. This is supported by the mean scores reported for items 13 and 14 after completion of the activities. Group one students report a mean score of 3.0 (1.155) concerning paper chromatography and 3.13 (1.147) for column chromatography. Group two students report mean scores of 4.69 (0.549) and 4.11 (0.711) for the same items respectively.

SURVEY PART 2: DESCRIPTION

Part 2 survey consisted of ten items and was designed to assess student attitude toward biotechnology (central goal number three, p. 11) and their intent to pursue science in their future studies or careers after completion of the course. The survey asks the students to respond to each item by ranking the item from one to five. A rating of one indicates that the student strongly disagrees with the statement. A rating of five indicates that the student strongly agrees with the statement. A comparison can also be made concerning any differences in reported values by group one students who participated in the

survey one year after completing AP Biology. See Appendix K for the complete text of Survey Part 2.

Table 3 - Results Of Survey Number Two

Item Number	1994-1995 Mean (SD)	1995-1996 Mean (SD)	Delta Value
1	4.25 (0.775)	4.46 (0.697)	0.21
2	4.13 (0.806)	4.23 (0.710)	0.10
3	4.50 (0.730)	4.38 (0.637)	0.12
4	3.56 (1.315)	3.88 (1.143)	0.32
5	3.88 (1.310)	3.69 (1.050)	0.19
6	3.38 (0.885)	3.62 (0.752)	0.24
7	3.44 (1.031)	3.92 (0.845)	0.48
8	4.75 (0.447)	4.65 (0.745)	0.10
9	4.13 (1.025)	4.54 (0.905)	0.41
10	4.06 (0.998)	4.35 (0.629)	0.29

Mean response value 1994-1995 : 4.01, standard deviation : 0.449
Mean response value 1995-1996 : 4.17, standard deviation : 0.367
Mean delta value (response change from 1994-1995 to 1995-1996) : 0.25, standard deviation : 0.13
N = 45

SUMMATION OF RESULTS FOR SURVEY 2

Survey Part 2 item numbers 1 and 3 deal with the students' perception of what biotechnology is and their ability to objectively and informatively view information involving biotechnology. Group one students report a mean score of 4.25 (0.775) and 4.5 (0.730), and group two students indicate a mean score of 4.46 (0.697) and 4.38 (0.637) for items 1 and 3 respectively. This data suggests that both groups one and two perceive that they are better prepared to make informed decisions concerning biotechnology than they were able to prior to completing the course activities

Item numbers 2, 6 and 7 question the students' attitudes toward biotechnology and biotechnical research after completing AP Biology. Item number 2 asks about their ability to objectively deal with information as a citizen

than they were previously. Group one students reported a mean score of 4.13 (0.806) for this item, compared to a mean score of 4.23 (0.710) for group two students, indicating student confidence in their ability to be objective concerning biotechnical research. The average delta value for both student groups was 0.9, which further shows that there was little change in the students attitudes concerning item 2 after being out of AP Biology for a period of one year.

Item numbers 6 and 7 focus more on the student attitudes toward research in biotechnology, item 6 asks about skepticism toward industrial biotechnology, and item 7 addresses skepticism about medical biotechnology. Both student groups one and two are hesitant to suggest that they are less skeptical of industry, and remain fairly neutral with reported mean scores of 3.38 (0.885) and 3.62 (0.752) respectively. They are a bit more kind to the medical field, perhaps due to the presumed altruistic nature of medical research, or that many of these students are considering careers in the medical arena. Group one students report a mean score of 3.44 (1.031), neutral in their skepticism, and group two reports a mean score of 3.92 (0.845) indicating that they agree that they are less skeptical than they were previously. These data show that students are open to biotechnology research of both industry and medicine, yet they will reserve judgment based on the information presented.

Survey items 4, 5 and 9 ask students to assess their futures in science education and post secondary studies. This is an important area to investigate as we should, as science teachers, encourage our most capable students to pursue science related careers. To do this, we should make our students aware of career opportunities in the sciences. This needs to be done while students are still in high school and prior to their making a commitment to a course of study as they enroll in colleges and universities.

Item number 4 asks the students if they feel better prepared to select an

area of post secondary study. Group one students reported they are still neutral after one year out of AP Biology showing a mean score of 3.56 (1.315) and group two students feel slightly more prepared with a mean score of 3.88 (1.143).

Item number 5 asks the student whether they would be more likely to enroll in science courses in their post secondary studies after completion of AP Biology. Both student groups one and two are between neutral and agreeing with this statement reporting respective mean scores of 3.88 (1.310) and 3.69 (1.050). The average mean for this item (3.79) suggests that the students are leaning toward the sciences for their future studies. The average delta value (1.18) shows that there is a range of responses from mild disagreement to relatively solid agreement with the item indicating that many of the students really have not fully decided on their future plans.

Survey item number 9 asks the student to consider whether they feel more prepared to undertake studies at the university level after the completion of AP Biology. One of the goals of an advanced science class such as Advanced Placement Biology is to help students feel prepared to compete at the next level of study as a result of their effort. Both student groups report a range of responses between agreement and strong agreement to the statement that, as result of the class, they believe that they are more prepared to succeed in their post secondary education. Group one students report a mean score of 4.13 (1.025) and group two students show mean score 4.57 (0.905). This is encouraging and good news for science education as those of us in the field attempt to encourage our better students to continue their studies in some field of science at a higher level. It can be noted that there are more female students enrolled in AP Biology than the other AP science offerings at our school. There is a need to increase the number of young women pursuing the sciences, and

the enrollment of female students in AP Biology is encouraging and their continued participation in advanced science courses should be solicited and fostered by science educators.

The purpose for item number 8 on this survey was to generally assess student perception of their competence in the laboratory after the completion of AP Biology. Since the activities are a major focus and attraction for those enrolling in the class it is important to ask whether the goal is being met. Both student groups show strong indications that this goal is being accomplished, with reported mean scores leaning well toward strong agreement. Group one students mean score was 4.75 (0.447)and group two reported a mean score of 4.65 (0.745).

The last item on survey part 2 asks students if they believe that plants can be utilized as an interesting tool in the biotechnology lab as a result of the activities described in this document. Both student groups report positive results. Group one students report a mean score of 4.06 (0.998) and group two students report a mean score of 4.35 (0.29). The standard deviation value for the group one students reinforces the convention that hands-on exposure to science is more beneficial than the alternative. The group two students were offered more opportunity to work with plants in hands-on activities than the group one students. It is valuable to see that both student groups believe that plants can be utilized in an interesting way in the lab. Many students find plants to be rather boring organisms by the time they reach high school and it is important to develop and maintain a positive status for plants in the science lab.

CHAPTER FOUR

CONCLUSION

Over the course of the last seven years I spent a significant amount time and energy developing materials, labs, activities and discussions/lectures using the information learned through the MSU/NSF Summer Institute. These activities and materials have been the foundation used to promote a new science literacy for my advanced biology students.

During the last two years I have presented the activities described in this document, and the necessary discussions along with them to four classes of students, two classes each year for the years 1994-1995 and 1995-1996. At the conclusion of these two years I have attempted to measure the success and impact that the participation in my Advance Placement Biology course has had on these students especially on their confidence in performing tasks on equipment that is used in the rapidly growing arena of biotechnology. Of equal importance, I have attempted to measure my students attitudes and perceptions of biotechnology/plant biotechnology and whether the exposure to methods and activities in my classroom have had an impact on those perceptions. I also tried to ascertain how these activities have impacted their decisions about their own science education in the future.

The students used various lab techniques, tools and equipment that are mainstays of today's biology laboratory. These included spectrophotometry, chromatography, micropipetting, buffers, tissue culturing and aseptic technique.

The students also used many other techniques that are not dealt with in

this document, for example; centrifugation, electrophoresis, protein analysis and DNA isolation.

The three central goals of this document are:

1. High school students can learn to use and apply laboratory skills and techniques that are employed in biotechnology:
   A. Chromatography
   B. Spectrophotometry
   C. Pipetting/Micropipetting
   D. Aseptic Technique
2. Students will be more likely to recognize plants as a biotechnological tool after the completion of the activities described within this document.
3. Students perspectives and attitudes toward biotechnology will be more positive after the completion of the activities described within this document.

The three goals set forth in this document address whether it is reasonable to expect high school students to learn techniques involving tools and equipment utilized in college and research labs in performing biotechnological studies. Secondarily, can it be expected that students be persuaded to see plants as an interesting and viable tool to be used in the biotechnology laboratory? Further, can it be shown that high school students be instructed and exposed to the techniques and tools employed by colleges and research institutions so as to establish a positive or informed view toward biotechnology as citizens in the new biotechnical age of society?

SUMMATION OF DATA ON CENTRAL GOALS

Central Goal : 1

The data (see Tables 1-3, pp. 34, 42) suggests that high school students

can learn to use and effectively apply the tools and techniques of biotechnology in the high school laboratory. The post activities survey was given to students immediately following their completion of AP Biology (group two 1995-1996), as well as group one students (1994-1995)surveyed one year after completion of the course and the stated activities in this document. The results of the survey support the hypothesis that high school students can master these skills. The data show that for each process indicated in the survey, students expressed a significant increase in their ability to use the equipment or perform the necessary procedures to complete the lab. The data show that both student groups one and two pre and post mean scores from the survey rose from the zero/little confidence range to between the fair/good confidence range for the group one students and between good/excellent confidence range for the group two students. The group one students indicated an overall mean increase on survey one from a mean response of 1.55 (0.412) before to a mean response of 3.52 (0.834) after. This is an increase in the mean score of 1.99 (0.662). The group two students mean level of confidence rose 2.48 (0.371) overall, from a mean response value of 1.7 (0.384) before to a mean response value of 4.18 (0.33) after for the sum of all items on survey one. Ninety five percent of all the students surveyed responded that overall they were more competent in the laboratory than they were previously.

Central Goal : 2

The students involved in this survey agree that plants can be utilized as an interesting method of study in the modern biology laboratory. This was a consistent response for both groups of students. Eighty-two percent of those surveyed indicated that they agree or strongly agree with the stated item. Only one student in the survey indicated that they disagreed with the stated item, the other sixteen percent remained neutral. Of those students who indicated a

neutral position, fifty seven percent were from the group one students who did not perform the tissue culture activity in a hands-on format. The data for the group one students suggests that students may be less likely to view plants as a biotechnological tool without the experience of a hands-on lab situation. Thirty one percent of the students in group one indicated that they were neutral or in disagreement with the view of plants being an interesting tool of biotechnology. In contrast, eighty nine percent of the students in group two, who did the tissue culture activity hands-on, indicated a positive attitude toward the use of plants, and the remaining eleven percent indicated a neutral position.

It is apparent that students can be instructed and exposed to plants in a manner such that they will view plants as a useful and interesting tool to be employed in the laboratory. The data also suggests that those students who work with plants in a hands-on situation will be more likely to view plants as a useful and interesting tool.

Central Goal : 3

Both groups of students indicate that they are better able to be objective in their evaluation of biotechnical issues in the media and be more open minded and informed, as citizens, than they were previously. Results from group one students on survey part 2 show an overall mean of 4.01 (0.449) and group two students an overall mean of 4.17 (0.367) for the same survey. The overall mean difference between their responses was only 0.246 (0.13), showing there was little change in their attitudes and perceptions toward biotechnology after being away from the classroom for one year.

The students were asked several questions concerning their attitude and understanding of biotechnology. There was no significant difference in the response mean scores between the two student groups. Both student groups solidly agree that they are better prepared to view biotechnology more

objectively as a citizen now than prior to the completion of the course. Both student groups are cautious, however, when it comes to trusting the research by both industry and medical fields, but they do remain neutral as opposed to more skeptical.

Both student groups' perception of their understanding of biotechnology are nearly equal in their mean scores from the survey part 2. This indicates they are more comfortable with their ability to be objective in their assessment of news and issues involving biotechnology than they were before. The data also suggests that even though the students involved in the survey are more literate and confident in their abilities, they still have uncertainty regarding their career paths for the future. Even if these students do not elect fields of science as their primary focus in life, they will carry these experiences and attitudes into their future role as citizens.

STRENGTHS, WEAKNESSES and SUGGESTIONS

The strengths of this study are threefold. I believe that the fact that the students were surveyed after leaving school for the year was beneficial. I felt I received an honest evaluation and opinion concerning the items on the interview survey. Students were able to reflect on their year of AP Biology without the specter of final exams and semester grades looming over their responses to the survey. Grades were submitted and the students were under no obligation to respond in any manner other than honestly.

The second strength that I see, is that there were two sets of students involved over the course of two years. This allowed for comparison over time in both continuity of presentation and conceptual retention of material and attitudes concerning biotechnology. Natural differences in presentation of activities existed from class to class and year to year, but the students involved came away from the experience with fairly consistent confidences and attitudes.

This suggests to me that not only is presenting the activities in this document to students important, but providing them with the opportunity to explore these techniques in hands-on experiences is even more beneficial in developing attitudes that they will carry with them as adults into the next century.

In the strengths of how one looks at what is accomplished in the classroom, one will often find weaknesses. Such is the case with the third consideration of this study's strengths. Throughout the course of the year the students involved in this study were evaluated on their mastery of the objectives and skills of the class which overlapped with the activities described in the text of this document. Instead of using the typical objective driven tests to assess the outcome of what had been presented, I chose to assess my students overall experience and its impact on how they perceive their abilities and competence in the area of biotechnology. This was the purpose of the exit survey part 1 and part 2. I view these survey results as the strongest indicator that what I am doing in the classroom is effecting students in a positive manner. The surveys showed that students gained in their confidence in laboratory skills, as well as their perception of their ability to address contemporary biological issues as citizens. This is what we strive for as biology educators: to develop students who are independent thinkers and problem solvers, as well as students who are better prepared to face the challenges of the new age of biotechnology. The biotechnology train is coming into town , and without the right ticket it will pass our future decision makers right by.

I was a bit disappointed that I did not receive one hundred percent of my student surveys back in the mail. I did receive seventy eight percent of those mailed, which is probably more than could be expected from a typical 'mail and return' survey. In one regard this could be viewed as a weakness, but I believe the previously noted positive aspects of this being a mailed, anonymous survey

out weigh the negatives of missing some survey responses.

If I could travel back in time armed with the advantage of hindsight, I would approach some things differently. I believe that I would have added an introductory pretest to see just how much some the very sharp students could recall from previous experiences. I would also use the pretest as post course evidence to show the students just how much was accomplished during the course of the school year.

Another area that I would investigate, if I were in a position to collect and analyze data regularly, would be the attitudes of female students toward biotechnology and their decisions to further their studies in the sciences after the completion of high school. I have always maintained solid numbers of female students in my advanced placement classes, many of whom indicate that they wish to study the sciences in their post secondary endeavors. It would be interesting to have the female students of my first four years of teaching AP Biology in another district complete the same exit survey that I sent to my female students from my current school. A longitudinal study of this sort would prove most interesting to me as a teacher, but the logistics involved would prove most frustrating since I am no longer in contact with most of my former students.

Without the advantage of hearing back from all of my students, I can still take a positive view of what I am accomplishing in the classroom based on the returned surveys reported here. It is apparent that students in an average Midwestern city can be taught the global tools of biotechnical research and be expected to come away from the experience with a positive and informed attitude toward biotechnology. This very well may be the foundation upon which our new biotechnically literate citizens will build the shape of society into the twenty first century.

What I have learned most from this experience is that I can, and do, make

a significant impact on the students that come away from my class each year. The data supports my thesis that my students can perform activities that are very challenging and become 'problem solvers' instead of 'problem doers' in the laboratory. I can expect that my students will develop as investigators using sound methodology. I can expect that my students will see that plants can be utilized in exciting ways in the lab. And I can expect that my students will consider themselves more prepared and informed citizens as they venture forward with their wide range of future endeavors.

I believe that what I am accomplishing with my students in the classroom is valuable, and I feel that it is important that other science teachers return to the classroom themselves to reacquaint themselves with where biology education is today and is headed tomorrow. This an ongoing process in which all science teachers should strive to make an effort to involve themselves. It does make a difference to our science students, not only in the mastery of needed skills to compete at the next educational level, but in the attitudes that they will call upon as they become voting, working and contributing members of our society.

APPENDIX A

Formal Lab Write Up

Each student is required to obtain their own quad rule laboratory notebook that will be maintained throughout the year. All formal lab write ups are to be prepared prior to the start of each lab and observations are to be directly recorded into the lab notebook using a blue or black ink pen. Recording on loose leaf paper is not appropriate, therefore all data tables are to be prepared in advance of the lab to facilitate the recording of data.

Your lab notebook is to be prepared neatly so that the instructor can read it easily. Entries should be concise and accurate and prepared in such a manner that the instructor can readily follow your procedural steps to assess their correctness. In the occasion of an error you should neatly strike out the error with a single line, it is therefore recommended that you keep a small ruler within your notebook at all times. The ruler will also serve you well as there will be numerous times where tables and graphs will be required.

The right hand side of the lab book should be used exclusively in the preparation of your formal lab write up and should begin numbering on the first page of the first lab write up. The left hand side of the page should utilized in the event of changes in the lab noted by the instructor, sample calculations, preliminary graphs or copies of charts required for the analysis of data.

The general arrangement of the lab notebook should begin with a table of contents page that includes; the experiment number, title, page number and date(s) of the experiment. It may look like the following :

Table of Contents

Exp. No.	Title of Experiment	Page No.	Date

There should be two to three pages left after the table of contents page. The remainder of the lab book is to be used solely for the purpose of preparing and writing lab reports. Each formal lab write up should include the following sections:

Experiment Number and Title

Each experiment should be given a title and be numbered sequentially as they are performed in class. The experiment title should reflect the objective of the lab, and appear at the top of the first page of the lab along with the number of the lab. The lab number should be placed at the top and center of each page of the lab.

Objective

Each lab should have some central goal(s) that are identified in several short sentences or phrases. These should be listed rather than written in paragraph form and should reflect the desired outcome(s) of the experiment.

Background

The background section will vary from lab to lab but should include information such as: textbook references, definitions from lecture/pre-lab, formulas or other relationships that will be employed during the experiment.

Procedure

The procedure should be divided into two sections, the first being A) equipment required for the lab, and second B) chemicals that will be utilized throughout the lab. The equipment list should include general lab items and a specific description of any special equipment that is required.

A description of how the experiment is to proceed should follow, and be entered as a step by step set of instructions that can be easily followed by another person of similar experience. Spaces may be left where calculations and data yet to be determined can be entered into the lab report as the

experiment is performed.

The procedure of the experiment is to be prepared ahead of time before you enter into the lab and begin your work. It may be necessary, on occasion, to leave blank space where the procedure cannot be written until after the experiment is actually performed.

Data and Results

All data, calculations, graphs and tables are to be entered directly into the lab notebook. Rough graphs or tables may be entered on the left hand page. If there is questionable data, possibly due to human error in the lab, it should be noted with explanation. This may be footnoted and referenced to "page opposite" so that adequate explanation can be provided.

Conclusion

All conclusions and the reasoning used in arriving at those conclusions should be stated in prose. This should also include a discussion of whether the experimental objectives were met, how the experimental data compares to the expected data, and error analysis of the experiment overall.

Figure 1 - Sample Grade Point Breakdown

Title/Objectives (5) ..... _____
Background (10) ..... _____
Procedure
    Materials (5) ..... _____
    Procedure (20) ..... _____
Data And Results
    Calculations (10) ..... _____
    Tables (10) ..... _____
    Graphs (10) ..... _____
    Error Analysis (5) ..... _____
Conclusion (25) ..... _____
Total (100) ..... _____

Appendix B

Solutions and Buffers

Worksheet Questions: Solutions and Molarity

1. How many grams of NaOH are needed to have one Mole?
2. How many molecules of NaOH would you have in question 1?
3. Describe how you would prepare a three molar solution of NaOH. Show all of your work and calculations.
4. How would you prepare 100 mL of a 0.5 M NaOH solution?
5. If you were to use 22.5 g of glucose ($C_6H_{12}O_6$) in preparing 500 mL of a glucose solution, what would be the solutions' molarity?
6. How many grams of K_2HPO_4 (dibasic potassium) are needed to make 100 mL of a 75 mM solution?
7. Define pH.
8. How do the following pH values compare: 10, 7, 4, 1?
9. What is a buffer? Chemically, how does a buffer perform its job?
10. Show how you would prepare 100 mL of phosphate buffer, pK = 6.9, at each of the following pH values:
    a) pH = 6.9, 0.5M; b) pH = 6.5 ,0.1 M;
    c) pH = 7.3, 75mM; d) pH = 8.0, 50mM
11. Another type of buffer, Tris (with Trizma HCl), have a different pK value than the mono/dibasic phosphate buffer. If you were given the gram molecular weights of Tris and Trizma HCl, how could determine the pK of this buffer?

Experiment: Solutions and Buffers

Objectives:

1. To accurately prepare solutions of specified molarity.
2. To calculate the ratio of weak acid-base pairs in preparing buffers of specific pH.
3. To employ serial dilution in determining the relationship between buffer concentration and buffering capacity.

Background:

Nearly all biological systems are buffered so as to maintain a stable pH that is optimum for the biochemical processes that occur within that system. It is no surprise then that in biological experimentation buffers are often required to perform the experiment.

You will be using a variation of the Henderson-Hasselbach relationship to aid you in preparing pH specific buffers;

$\Delta = pH - pK = \log \alpha/1-\alpha$

Δ = the difference in pH needed and the pK of the buffer

pH = the pH needed in the final solution

pK = the anti-log of the buffer system dissociation constant

α = the fraction (%) of the base required for the buffer

$1-\alpha$ = the fraction of the acid required for the buffer

The value of delta is calculated, and the value of alpha is obtained from the table of various delta value and their corresponding alpha values. The alpha (base %) and 1-alpha (acid %) are then used to determine the volumes of each needed top prepare the buffer.

Procedure:

Unique Materials and Chemicals:

1. Potassium Monobasic
2. Potassium Dibasic
3. Electronic Balance
4. Electronic pH meter

Procedure:

Part A

1. Prepare the following solutions (50 mL each):
    A. Potassium Monobasic: 0.25 M, 0.1 M, 50 mM
    B. Potassium Dibasic: 0.25 M, 0.1 M, 50 mM
2. Parafilm, label and store.

Part B

In large testtubes:

1. Prepare 10 mL of 0.25 M phosphate buffer, pH of 6.9
2. Prepare 10 mL of 0.1 M phosphate buffer, pH of 6.0
3. Prepare 10 mL of 50 mM phosphate buffer, pH of 7.6
4. Check and record the pH of all three buffers.
5. Determine the percent error in the buffers prepared.
6. If any of your buffers are more than 0.1 pH units from the expected value, adjust the pH to within 0.1 pH units.
7. Parafilm, label and store.

Part C

1. Prepare a serial dilution of the 0.25 M buffer to obtain approximately 10mL of the following dilutions:10X, 100X, and 500X
2. Prepare a sketch showing the volumes of buffer and water that you transferred in the preparation of these dilutions.

3. Check and record the pH values of each of your dilutions, as well as, the water that is being used for the diluting.
4. Check and compare the buffering capacities of each of the buffer samples used in Part C, 1X, 10X, 100X and 500X. Do this by adding 0.1 M HCl in 0.5 mL alloquats, mixing gently and checking the pH of the buffer after each addition of HCl. Continue until the pH of the buffer reaches a value of 2 pH units from the starting pH. Record and graph your results.

Data and Results:

Be sure to show all of your calculations, tables and graphs. Each of these should be titled for reference purposes in your conclusion.

Conclusion:

Revisit your objectives and address whether you met each.

1. Discuss the preparation of the buffers and your percent error analysis explaining where you had difficulty.
2. What do you know about a buffer and its ability to perform when its prepared so that the pH is equal to the pK for that buffer?
3. What happens to a buffer as it:

   A) becomes diluted, and B) has acid or base added.

Instructor notes:

In order for students to perform this activity they will need to be instructed in the following areas;

1. preparing solutions from dry reagents
2. pipetting
3. use of the electronic balance
4. use of an electronic pH meter (or pH paper)
5. use of the Henderson-Hasselbach equation

$$\Delta = pH - pK = \log \alpha/1-\alpha$$

6. given the following table to determine base % and acid %

Figure 2 - Logarithmic Conversion Table for Δ and α Values

Δ	α	Δ	α	Δ	α	Δ	α
0.00	.500	0.00	.500	-1.00	.091	1.00	.909
-.05	.471	.05	.529	-1.05	.082	1.05	.918
-.10	.443	.10	.557	-1.10	.074	1.10	.926
-.15	.415	.15	.585	-1.15	.066	1.15	.934
-.20	.387	.20	.613	-1.20	.059	1.20	.941
-.25	.360	.25	.640	-1.25	.053	1.25	.947
-.30	.334	.30	.666	-1.30	.048	1.30	.952
-.35	.309	.35	.691	-1.35	.043	1.35	.957
-.40	.285	.40	.715	-1.40	.038	1.40	.962
-.45	.262	.45	.738	-1.45	.034	1.45	.966
-.50	.240	.50	.760	-1.50	.031	1.50	.969
-.55	.220	.55	.780	-1.55	.027	1.55	.973
-.60	.201	.60	.799	-1.60	.025	1.60	.975
-.65	.183	.65	.817	-1.70	.020	1.70	.980
-.70	.166	.70	.834	-1.80	.016	1.80	.984
-.75	.151	.75	.849	-1.90	.012	1.90	.988
-.80	.137	.80	.863	-2.00	.010	2.00	.990
-.85	.124	.85	.876				
-.90	.112	.90	.888				
-.95	.101	.95	.899				
-1.00	.091	1.00	.909				

Time Requirements:

Solutions pre lab and solution preparation: 2.0 periods

Buffer preparation: 1.5 periods

Serial dilution: 1.0 period

Appendix C

Chromatography

Experiment: Paper Chromatography

Objective:

1. To investigate the separation of dyes and pigments using paper chromatographic technique.
2. To identify the number and type of pigments in a plant sample.

Background:

Chromatography is a common laboratory tool that is used in biomolecular and chemistry labs around the world. It is most often used to isolate a particular compound, and occasionally to clean up a sample prior to further use. Chromatography takes a variety of forms, paper chromatography being the earliest and simplest. Other forms include; column chromatography (ion exchange, reverse-phase), thin layer chromatography and HPTLC (high performance thin layer chromatography). We will investigate the use of reverse-phase chromatography in the next lab.

The process of chromatography involves using the chemical differences between solutes in a solution to separate the various solutes for discovery or analysis. Just as we can separate the particles of sand and salt in a water solution by filtration and evaporation, we can employ chromatography to separate solutes of similar solubility from each other in a solution.

In paper chromatography, we rely on the differences in solubility of the solutes in the solvent. A dried spot of solution is applied toward the base of a strip of chromatography paper. The tip of the paper is allowed to

contact a pool of solvent in the bottom of a chromatography chamber, or testtube, as the paper wicks the solvent up the length of the paper it passes through the dried solute spot dissolving the various solutes and carrying them up the strip of paper. Those solutes that are most soluble will travel farthest and fastest and the others will trail behind in order of solubility. If needed a comparison of the solutes rates of movement along the paper can be determined using the distance traveled by the leading line of solvent as a reference.

The solutes that are separated will leave distinct lines of color where they are deposited, hence the name chromatography.

Procedure:

Unique Materials and Chemicals:

1. Black Vis-a-Vis Marker
2. Paper Chromatography Chamber
3. Whatman Chromatography Paper
4. Chromatography Solvent (Part B)

   90% Acetone 10% Pet. Ether
5. Several plant leaves from your choice of plant (Part B)

Procedure:

Part A

1. Obtain your materials
2. Cut your Whatman paper so that it has a pointed tip on one end and is just long enough to be suspended in the chamber without the tip touching the bottom, or the paper contacting the sides.
3. Prepare a concentrated dot of solute (Black Vis-a-Vis Marker) at a point approximately 1.5 cm from the tip of the bottom of your paper. Do this by touching the tip to the paper with the marker several times

being certain to dry the spot between applications.

4. Transfer enough water to the chamber so that it is no more than 1.0 cm in depth.
5. Suspend your prepared paper with sample in the chamber being certain that the tip is immersed in the water and the dried marker spot is above the level of the water.
6. Allow the solvent (water) to travel to within 2.0 cm of the top of the paper, at which time remove the paper and allow it to dry.
7. Record your observations in your lab book.

Part B

1. Repeat the procedure for Part A with the following changes:
    a) Replace the marker spot with a plant leaf smear or extract of the leaf "juice". This can obtained by grinding the leaf sample with a mortar/pestle.
    b) Replace the water with chromatography solvent

Data and Results:

Prepare a diagram that shows your experimental set up and label it accordingly, Part A by the color and Part B by the pigmentation color. You should prepare a sketch of your final chromatographs for both Part A and B.

Research the pigmentation in plants and hypothesize the identity of the pigments separated in Part B.

Conclusion:

Revisit your objectives. Have they been met?

1. Compare the solubility of the dyes that are in the black marker.
2. Report on the pigments that were identified in the plant sample.
3. Compare and contrast the black marker dyes to the plant pigments. Why were different solvents used in this activity?

Experiment: Food Dye Chromatography

Objective:

1. To employ reverse-pahse column chromatography to separate the dyes used in the making of grape drink mix.
2. To use the skills learned from separating drink mix dyes to separate the dyes from a food sample of your group's choice.
3. To reinforce the skills of:
    a) solution preparation
    b) pipetting
    c) preparing a formal lab report

Background:

Reverse-phase chromatography is an excellent tool for separating dyes and pigments found in foods and organisms. It relies on the differences in the dyes or pigments polarity. A column, consisting of a silica gel matrix bonded with Carbon Octadecyl (C_{18}), is prepared for use (conditioned) and then loaded with a sample of a solution containing solutes from the material that is to be tested. A series of elutions are run through the column each with a different polarity based on the ratio of water to organic solvent in the hopes of separating the sample into its constituent dyes or pigments. The solute(s) load on to the column due an affinity relative to the polarity of the column. If an eluent is run through the column that will dissolve one or more of the solutes the solute(s) soluble at that polarity will elute through the column with the eluent. If the solutes loaded onto the column are close in their polarity the separation is more involved. A polarity range can be established, and an elution gradient used to separate the dyes or pigments. By collecting the eluate in separate containers an estimate of the relative amounts used in the making of the product.

If you are testing a food sample, your results can be compared with the

products ingredient list found on the package.

Procedure:

Unique Materials and Chemicals:

1. 13mm X 100mm testtubes and testtube rack
2. one syringe, 3mL or larger
3. one reverse-phase C_{18} mini prep chromatography column
4. Grape drink mix powder
5. Distilled water
6. Food sample, group choice
7. Ethanol 95%
8. Isopropanol 99%
9. Other solvents may be required in the analysis of the group specific food sample

Procedure:

Part A

1. Obtain all of your materials
2. Prepare the following solutions:
    a) Drink mix powder: 100mL of a 0.75 g/100mL solution
    b) Ethanol: 70% and 20% solutions, 50mL each
    c) Isopropanol: 20% solution, 50mL
3. Condition your column with 1mL of 70% ethanol(EtOH):
    - Draw 1mL of EtOH into the syringe and place it into the top of column cartridge and slowly drip the EtOH onto the column until the last drop barely pools on the top of the column. From this point on the column should remain wet until the conclusion of the activity.
2. Load the column with 1mL of drink mix solution:

- Draw 1mL of the sample into the rinsed syringe and slowly drip the solution onto the column.
- Continue until the last of the 1mL barely pools on the top of the column. You should now see a band of grape food dye at the top of the column. This is your sample!

3. Elute the column with 3-4mL of 20% EtOH:
   - Continue at steady drip rate collecting the eluate in a testtube until a band of color moves through the column and is ready to be collected. At this point switch testtubes and collect all of the eluate that contains the first dye as it passes through the column.
4. Determine the amount of eluent collected in the first two tubes and record your observations.
5. Repeat steps three and four using 3-4mL of 20% Isopropanol to elute through the column and collect the second colored dye that comes off the column.
6. Wash the column after you have collected both of the dyes by eluting with 3mL of 70% EtOH and then 3mL of distilled water, repeat this step two times.

Part B

1. Prepare your food sample for use with the mini prep column by using one to two mL of your sample to load the dye onto the column as you did in part A.
2. a)If your sample does not load using the procedure in part A you have to adjust the polarity of the column before you can load your sample.
   b)If you are using a sample that is bound to a solid food item, you must first remove the dye from the food. This is best accomplished by soaking/swirling the sample in water to remove the dye. Then load the

sample as in part A.

3. Proceed as you did in part A. You will need to work by trial and error to separate the food dyes that are in your sample. Be sure to record all of the elutions with their volumes and concentrations that you use and collect the colors in separate testtubes.
4. When you have collected all of the dyes that you can, refer to the label from your product sample for comparison and confirmation.

Data and Results:

Show all of your calculations for the preparation of your solutions that were needed to complete the lab.

Prepare tables to show the volumes of each eluent that was collected and what food dye collected with each. Report the comparison data from the label of the product as well.

Conclusion

1. Discuss the process of chromatography and what it can be utilized for in the laboratory.
2. Discuss the results of the separation of the dyes in your food sample, include a comparison of your experimental and expected results.
3. Discuss any difficulties you encountered during the lab

Instructor Notes:

The mini prep columns can be purchased from J.T. Baker Company:

J.T. Baker Company

222 Red School Lane, Phillipsburg NJ 08865

1(800)-582-2537

You may be able to receive a complimentary supply, due the nature of their use, with a phone call to the customer service department . The syringes can be obtained through any number of supply companies, or even from a local

pharmacy.

Examples of some good sources of food dyes are M&M's, Trix cereal, Spree, Runt's candy and various soda pop beverages. When using a solid sample the students should be careful not to dissolve more than just the colored covering. The underlying candy can easily gum up the column making for difficulty in completing the lab.

If you have a group of very capable students who need a challenge, provide them with an orange food sample containing Yellow Dye #5. After much experimentation they may come to the conclusion that the orange dye cannot be separated into yellow and red, or they may discover that Yellow #5 is actually orange! The mini prep columns they can reused for several years depending on the material that has been used in them. Eventually they will be too dirty or gummed to be effectively used.

Time Requirements:

Paper Chromatography: 0.5 period preparatory
1.0 period laboratory

Column Chromatography: 2.0 periods

Appendix D

Spectrophotometry

Objectives:

1. Learn to read and operate a spectrophotometer.
2. Learn to apply the Beer-Lambert equation.
3. Reinforce the skills of collecting, analyzing and graphing data.
4. Use the technique of serial dilution in the preparation of samples to be tested.
5. Apply the use of spectrophotometric technique to determine the amount of protein in samples of commercial pet food

Background:

A spectrophotometer is an instrument that passes a light beam of specific wavelength through a sample solution that is either an unknown, or contains a material that can be detected by the Absorbance of light by the material in question. The latter method can also be used in conjunction with the mathematical Beer-Lambert equation to determine an unknown concentration of the substance in question.

A spectrophotometer consists of a electromagnetic radiation source that can be set to emit radiation in range of wavelengths generally found to span most of the visible spectrum (approximately 350nm to 700nm). Once the desired wavelength is set the light is passed through sample solution contained in a small specially designed testtube (approximately 13mm x 100mm). As the light passes through the sample (approximately 1mm thick) some of the light is absorbed by the sample and the remaining light passes on through and strikes a detector. The intensity of the light striking the detector is then transferred to an

analog read out dial, or displayed digitally on the front of the spectrophotometer.

In order to accurately describe the amount of light actually absorbed by the chemical in question it is necessary to account for the background Absorbance by the remaining portion of the solution that you are not interested in. To do this you must prepare a solution without the chemical in it that can be used to adjust the reading of the meter to show a reading of 100 percent transmittance of light. It is also necessary to set the Absorbance of the meter to zero before starting. With the spectrophotometer "zeroed" you can now insert the sample and read the meter and determine the comparative transmittance (or Absorbance) of light by your sample. It is important to note that the meter looks very different in terms of transmittance and Absorbance, this is because the Absorbance represents an anti logarithmic function of transmittance: A = -log T.

To utilize this data you must have some information about the chemical that you are investigating. A constant value that is characteristic of the chemical's Absorbance at that wavelength is called the chemicals' extinction coefficient. You also need to know how much of the chemical solution the light is passing through, the thicker the layer of solution, the greater the Absorbance due to the higher number of molecules. The Beer-Lambert equation shows the relationship between these values:

$A = elC$ where:

A = Absorbance

e = extinction coefficient

l = length of light path

C = the concentration of the chemical in question

Procedure:

Unique Materials and Chemicals:

1. spectrophotometer
2. spectrophotometer sample tubes (5), or 13mm x100mm TT
3. Eriochrome Dye 400mg/mL

Procedure

Part A

1. Gather all of your materials, turn on your spectrophotometer and set the wavelength to 528nm
2. Prepare a stock supply Eriochrome Dye at 400mg/mL
3. Prepare the following serial dilutions of your stock Eriochrome solution using distilled water

   a) 0.1 b) 0.01 c) 0.001 d) 0.0005

4. Transfer 4mL of each of these dilutions into separate spectrophotometer sample tubes
5. Transfer 4mL of distilled water into your fifth sample tube this will be your blank for setting the meter at 100 percent transmittance
6. Be sure to label each of your sample tubes with the tape at the very top of the sample tube so that it does not interfere with the light emission from the spectrophotometer
7. Use the spectrophotometer to determine the transmittance (or Absorbance) of each of your samples and record the results in table form
8. Graph your Absorbance of the samples as a function of concentration
9. Prepare a conclusion:
   a) relationship between Absorbance and concentration
   b) calculate the extinction coefficient for Eriochrome
   c) Use the Beer-Lambert equation to determine the experimental concentration value for your samples

d) compare your calculated values for concentration to the actual concentration of your serial dilutions

Part B

Using samples from pet food products of your choice, you will determine the amount of protein in the sample and compare it to the known value reported on the nutritional panel of the pet food container.

Procedure:

Unique Materials and Chemicals-Additional

1. BCA Reagent A and B
2. NaOH solution 0.1M
3. pet food samples (minimum two per group)
4. Mortar and Pestle

Procedure:

Day One

1. Gather materials and prepare 0.1M NaOH solution
2. Grind one pet food pellet into a fine powder using mortar and pestle.
3. Measure 0.05g of pet food and dissolve it into 10mL of 0.1M NaOH. Repeat this for each different sample to be tested.
4. Separate the residue from the solution by either centrifugation or filtration to collect the clear supernatant of filtrate. Label your samples.

   Note: It is important to keep track of your dilutions throughout the lab so you can compare grams of protein in percent by weight with the percentages listed for the pet food on their respective containers at the completion of gathering your data.

Day Two

5. Prepare a working assay of BCA Reagent by adding 49mL of reagent A with 1mL of reagent B (50:1)

6. Mix 0.1mL of your sample supernatant(filtrate) into 2mL of BCA assay and allow to incubate at room temperature for 30 minutes
7. Turn on your spectrophotometer, and set to 562nm
8. After 25 minutes: zero the spectrophotometer and set the transmittance at 100 percent using 2mL of BCA assay and 1.1 mL of distilled water as your blank
9. At the end of incubation, mix your sample and transfer 0.1mL to a 13mm x 100mm TT containing 2.0mL of BCA assay and 1.0mL of distilled water
10. Record your results, and repeat this procedure for all of your samples to be tested
11. Calculate the concentration for your samples using the extinction coefficient provided by your instructor, the length of the light path and each sample. Note: You may need to calculate an extinction coefficient using samples of known protein concentration.

Data and Results:

Include all of your calculations, graphs and tables, and percent error calculations. Include the comparison of percent weight of your protein sample to that of the reported value from the manufacturer in your percent error analysis.

Conclusion:

Part A

Be sure to address Procedure items eight and nine, along with your percent error analysis.

Part B

Revisit your objectives and report on your success in this lab and how your results compare to that of the manufacturer. Discuss how different they are

and possible reasons as to why the results differ.

Instructor Notes:

BCA Reagent is available from Pierce Chemical Company:

Pierce Chemical Co

3747 N. Meridian RD.

PO Box 117

Rockford IL 61105

1-800-8-Pierce

They have a strong reputation for helping out educational institutions and will likely provide free samples of the reagent to requesting individuals who are instructors. The BCA Reagent is stable for about two years maximum, so you should not order any more than you will require in a year.

If you would like to have your students determine the extinction coefficient for the BCA Reagent with protein your can have your students prepare solutions of Bovine Serum Albumin-BSA, (which is included with the BCA Reagent) that range in concentration from 5.0 ug/mL to 50ug/mL and prepare a graph of the Absorbance as a function of concentration. Unknown concentration values that fall between these concentrations can be determined by interpolation on the graph when the experimental values for Absorbance are obtained.

Time Requirements:

Part A and B: 1.0 period Preparatory

Part A: 1.0 period laboratory

Part B: 3.0 periods laboratory/analysis

Appendix E

Plant Tissue Culturing

Objectives:

1. Learn to employ aseptic technique
2. Understand the principles of sterilization
3. Organize, collect and record data over an extended period
4. Observe the effects of two hormones on the differentiation of cells in a plant leaf tissue culture

Background:

Plants grow and develop from a single fertilized cell just like you and I. The fertilized seed grows rapidly making new cells by asexual reproduction. Due to the presence of hormones within the cells, the developing cells begin to differentiate, that is to say, they begin to form specialized cells that develop into different types of tissue. These tissues will develop into the roots, shoots and eventually the leaves and remaining parts of the plant. The information for these tissues can be found encoded in the chromosomal DNA of each plant cell. When a cell is first beginning to divide and make new cells this information is being copied and passed along to all of the new cells that result from the division of the original cell. In the presence of the plants hormones this information code is accessed and turned on. This is the information that causes cells to elongate downward and form roots, and other cells to elongate upwards and form the shoots that will form the stem and eventually the remaining cells that comprise the entire plant.

It is conceivable that in the absence of one or more of these hormones the differentiation of cells during the the development of tissue would cause the

plant to produce one type of tissue and not another. It is also conceivable that in the absence of all the growth hormones during this development period would result in the formation of cells without differentiation into specialized tissue. It is these very ideas that we will investigate during this activity!

Procedure:

Unique Materials and Chemicals:

1. Autoclave (large pressure cooker)
2. Tissue Culture Tubes and Caps (8 per group) - Sterile
3. Scalpel (2 per group - Sterile
4. Long Forceps (2 per group) - Sterile
5. Tissue Culture Media A and B
6. Sucrose 30g per 1 liter of media
7. Agar 8 g per 1 liter of media
8. Household Bleach Solution(1% and 10%)
9. Ethyl Alcohol (70% and 95%)
10. Detergent Solution (1%)
11. Sterile Distilled Water
12. Hydrochloric acid 1 M, Sodium Hydroxide 1 M to adjust pH
13. Sterile Petri Dishes as needed
14. Aluminum Foil (for sterilizing materials)
15. Chrysanthemum Leaves
16. Hot Plate
17. Electronic pH Meter
18. Heat protective gloves
19. Transparent transfer shield

Procedure

1. Divide your group into teams that will work on various phases of the

preparation of this lab. One person will work with individuals from other groups to prepare the culture media for the class. Another person will work with other class members to prepare all of the hardware and distilled water for sterilization, and start the first run of the autoclave. The remaining members of the group will gather the remaining materials and prepare the needed solutions.

2. Gather all your materials and prepare solutions:
    * 100 mL of 1% Bleach (store in a squirt bottle)
    * 200 mL of 10% Bleach
    * 100 mL of 1% tap water and detergent(Joy works well)
    * 100 mL of 70% ethanol (from 95% stock ethanol)
    * 25 mL of 1M HCl
    * 25 mL of 1M NaOH

    Label each solution accordingly and parafilm each container and store use during the next lab session.
3. Media preparation. Prepare 1 L each of Media A and Media B.

    For each liter of media :
    * transfer 800 mL of distilled water into a 2 L Ehrlenmeyer Flask
    * place on a hot plate and heat (do not boil)
    * add the contents of one Media A package into one flask and Media B into the other. Rinse each package with water and add to the correct flask to ensure transfer of all of the media powder, mix each.
    * weigh out a mass of 30g of sucrose (table sugar) per flask and add to each flask, stir until dissolved
    * adjust the pH of each flask using 1 M HCl or 1 M NaOH until a pH of 5.7 is obtained
    * weigh out and add 8g of agar to each flask and stir until

dissolved (remember not to boil this solution)

4. Prepare eight culture tubes for each lab group, four of media A and four of media B. Transfer 15 mL of the appropriate media into each assigned culture tube, using heat resistant gloves, and cover with an autoclavable cap.
5. Autoclave the prepared culture tubes for 15 minutes at 15 pounds per square inch pressure.
6. After autoclaving, remove the media tubes and allow to cool at a 45 degree angle so as to form an agar slant as the media solidifies.
7. Prepare distilled water and hardware for sterilization.
    * transfer 100 mL of distilled water into each of three 250 mL flasks and cover securely with aluminum foil
    * wrap two each of forceps and scalpels with aluminum foil
    * autoclave the distilled water and hardware for 15 minutes at 15 pounds per square inch pressure

Procedure for Plant Tissue Culture

1. Clean the work area thoroughly with the 1 % bleach solution. Wash your hands thoroughly with soap and water.
2. Choose four healthy chrysanthemum leaves and place into a clean 250 mL beaker
3. Add the detergent solution and wash by gently shaking the beaker periodically for three minutes
4. Pour off this solution and rinse well with tap water
5. Add 50 mL of 70% ethanol and agitate gently for one minute drain off the ethanol into a waste container
6. Add 100 mL of 10% bleach solution and agitate gently for two minutes

NOTE: The samples of leaf tissue are now considered sterile and aseptic

technique should be employed for any procedure henceforth. Use only sterile containers. Instruments be autoclaved and dipped in 95% ethanol and flamed between each use. Extreme caution should taken to keep the open beaker of 95% ethanol well away from the burner used for flaming the instruments or tips of culture tubes. Subsequent procedures should be carried out under the transparent transfer shield.

7. Pour off the bleach solution, using the sterile blade of a scalpel to block the leaves from exiting the beaker
8. Perform three consecutive washes with sterile distilled water. Add 100 mL of sterile distilled water and agitate gently for two minutes, and pour off the water. Repeat three times.
9. Transfer the sterile leaves to a sterile petri dish. Using a sterile scalpel and forceps, cut the leaves into 1 cm square pieces until you have eight squares. Avoid using the central vein of the leaf.
10. Aseptically transfer each leaf square into one of the slant media tubes so that you have four tubes of each media A and media B containing one leaf square
11. Reflame the tip of each tube before recapping and seal the cap to the tube by wrapping with parafilm
12. Place the tubes 30 cm under fluorescent lighting
13. Observe and record your cultures daily for the first week. Remove any tubes that exhibit contamination.
14. Record your observations weekly of remaining tubes and continue to remove any that show contamination

Data and Results:

Include a table designed to record data on the progress of each your leaf cultures.

Show calculations for the preparation of all solutions.

Media A and Media B contain different plant growth hormones. Research the following terms and indicate your sources of information:

hormone

Indole Acetic Acid

Giberellic Acid

Kinetin/Cytokinetn

root/shoot/callus growth

in vitro

in vivo

Conclusion:

Summarize your results of each of the culture tubes you prepared. Distinguish any differences that were observed in the growth of cultures in media A versus media B plants. Hypothesize as to the hormones that were present in media A and media B, support your answer with your observations.

Discuss the difficulties in performing this procedure and what you would change if you were to do this lab again.

Compare the role of hormones in the development of plants to that of the role of hormones in the development of a human zygote into a human embryo.

Instructor Notes:

It is strongly recommended that prepackaged media be purchased from a biological supply company, such as Carolina Biological Supply or other reputable company. This will save large amounts of time and better ensure success with the culturing of tissue.

An autoclave is not necessary for sterilization of materials. Most high school labs use a large pressure cooker that can be purchased from most suppliers. It is necessary that materials be sterilized or this lab will never work.

Contamination of the culture tubes will be the biggest obstacle in the way of your students success. Transfer boxes are suggested but are not necessary. These transfer boxes need not be elaborate to work well. They may consist of little more than molded plexiglass with heavy plastic wrap sides. In short, anything that reduces the flow of air around the sterile petri dishes and culture tubes will help to reduce the risk of contamination.

There are many protocol available for tissue culturing using many different plant tissues. It is advisable to contact your favorite biological supplier and inquire as to what media is available for use with the plants that you have access to. Some examples of plants that are use include: Lettuce, Broccoli, Cauliflower, Tobacco, Carrot, Hosta, African Violet, Chrysanthemum (as is used in this protocol) and even Venus Fly Trap. Protocol can also be either purchased or acquired through various sources, such as, The American Biology Teacher Magazine, Access Excellence (InterNet), and your local college or public library.

In order to reduce the amount of time that is devoted to this lab the instructor may choose to prepare some of the solutions or media in advance for the students. It is likely, in any case, that the instructor will need to perform some sterilizing for the classes due to the length of time it takes to autoclave materials. Students will need to be instructed on the methods and importance of aseptic technique in tissue culturing. Remind students as to the flammability of ethanol and the importance of being safe in the lab.

Time Requirements :

Set up and sterilization of materials: 1.0 - 1.5 periods

Preparation of tissue culture: 1.0 periods

Observations: Ongoing for approximately four weeks

Appendix F

Environmental Impact on Seed Germination

Objectives:

1. To design an experiment with proper variables and controls
2. To observe and collect data on the growth of an organism
3. To determine the effects of an external factor on the germination and subsequent growth of a plant *B. rapa*
4. To construct a Self-Wicking Mini Growth Chamber

Background:

Plants, as in all eukaryotic organisms, contain the genetic information to direct their cells to produce the various tissues and structures. The influence of these hormones is expressed early on in the development of a plant as it germinates in the soil. Different hormones initiate the expression of different genetic information that cause the plant to produce tissue that differentiates into roots, stems, leaves and eventually flower buds. Under normal conditions the germination and differentiation of tissue proceeds as directed by the species genetics and the plant develops normally.

Plants and other organisms evolved to their current condition without the influence of modern society and the new environmental pressures from the age of technology and pollution. Knowing what some of these factors are, we can design controlled experiments that allow us to observe first hand how a plant responds to particular environmental factor.

To facilitate this, we will use a cultivar from the Cruciferae family of plants that includes broccoli, cabbage, mustard and rutabaga, to name just a few. The plant we will employ is named *Brassica rapa* (*B. rapa*) after its ability to reach

maturity in a relatively short time. *B.rapa* was developed in the 1980's by Dr. Paul Williams (et.al) at the University of Wisconsin-Madison. It was genetically selected and bred over a number of years to produce flowers and mature seeds in shorter and shorter cycles. The result is a plant we today call the Wisconsin Fast Plant, or Rapid Cycling *Brassica rapa*, often called RCB's or Fast Plants for short. The RCB's are useful because we can readily grow them in the lab, and they run a full cycle from seed to seed in just 36-40 days.

Your group will need to decide on an environmental factor that you wish to test for and determine the methods you will use to expose your plant to the factor you choose. The vessel in which you grow your plants is called a Self-Wicking Mini Growth Chamber. The value of this type growing apparatus is its ability to be left with minimal care for days at a time and be safe under fluorescent lighting 24 hours per day. The chambers are also small so that it allows you to set up control and variable plants without requiring to much space under the light banks.

Procedure:

Unique Materials and Chemicals:

1. Self-Wicking Mini Growth Chamber
   - *RCB seeds, two per growth tube
   - *plastic margarine tub, 8oz size works best
   - *1.5mL microcentrifuge tubes (growth tube), one per each control and variable plant to be grown
   - *cotton string, for wicking water to the seeds
   - *10-10-10 NPK fertilizer
   - *peat/vermiculite mix, planting media
2. Light banks, 40 watt fluorescence (provided)
3. all other materials will vary dependent on the procedural design of

your experiment, these must be approved by the instructor prior to the start of your experiment

Procedure:

After you have received clearance from your instructor:

1. Gather all of your materials
2. Prepare your Self-Wicking Growth Chamber, note that you will need to cut the tip of the microcentrifuge tube off to allow for the wicking string to pass through the tube
3. Prepare your control in the following manner:
   *thread the string (moistened) into place first, you may need to knot the the string where it passes through the hole in the bottom of the microcentrifuge tube
   *fill the tube one third full with moist media (soil)
   *place two fertilizer pellets on media
   *finish filling to the top of the tube with media
   *place two RCB seeds on top, cover lightly with moistened media
4. Prepare and set up your variables you will be testing, and your control, so that the growth tubes can be inserted into a cut hole in the top of the margarine container and remain suspended with the string laying in the water, or test solution, in the bottom of the margarine tub, the string will wick the water up and supply the plant with needed water for germination and growth (see appendix L)
5. Be sure to include procedures for the preparation of your experimental design apparatus and/or solutions

Data and Results:

This is an activity that you will need to watch for several weeks, therefore, you will need to prepare a data table that will allow for the recording of

observations over an extended period of time. The first week of observations will be critical as the seeds begin to germinate. After the first week you will only need to record observations every third day, although this may vary depending on what factor you are testing for.

If you have prepared solutions or apparatus for your experimental set up include any calculations used in the process.

Conclusions:

1. Were you successful in growing your RCB's? If yes, describe your results. If no, describe the steps that were taken to correct any problems.

2. Did you observe any effect of your variables on your test plants? How did you come to your conclusions? How was this measured?

3. Make a generalized statement concerning the effect of the environmental factor you tested for on the germination or growth of your plants.

4. If you wanted to find out more about the effects of your experimental factor on plants, how you change your design to continue testing further?

Instructor Notes:

This lab activity allows students to pursue their own ideas about what and how environmental factors impact the germination and growth of plants. I have found that students can be very creative in this lab and have great success with a little common sense guidance from the instructor. Some ideas of possible variables are:

a) varying pH of the watering solution, usually from the top to simulate acid rain

b) testing the effect of electromagnetic radiation in close proximity to the germinating plants

c) testing the effect of various concentrations of a pesticide, herbicide or fertilizer

d) testing the effects various wavelengths (colors) of light on the germination and growth of the plants

Wisconsin Fast Plants, RCB's, have been in use in classrooms since the late 1980's and have been adapted for use at nearly every level of science education. Dr. Paul Williams coordinates and runs workshops that assist science and elementary teachers alike in using Fast Plants in their classrooms. These workshops are useful, but not necessary for working with Fast Plants.

The Fast Plant seeds are available through Carolina Biological Supply, along with a host of genetic mutants and the accompanying lab directions to guide a new Fast Plant user through a lab. All of the materials for growing Fast Plants can be purchased through Carolina Biological Supply, including the watering system developed specifically for Fast Plants. I have found that the watering system gets bulky and really limits the possibilities for students to design their OWN experiment. I have also been able to eliminate the use of copper sulfate as an algae inhibitor, this system does not seem to require one! This is why I designed and recommend the Self-Wicking Mini Growth Chamber for this lab.

I do recommend that teachers also use the Fast Plants in their classes for the study of plant genetics and anatomy. The students really enjoy taking responsibility for the growth of an organism.

Time Requirements:

Set Up of controls and variables: 1.0 - 2.0 periods

Observations: ongoing for at least two weeks

Appendix G

Plant Pigments: Chromatography and Spectral Analysis

Objectives:

1. To use both paper chromatography and spectrophotometry to analyze the pigments extracted and separated from a plant leaf
2. To use skills from previous labs and apply them in solving a new problem
3. The student will learn that plants use a variety of pigments
4. The student will investigate the correlation between pigment color and wavelength Absorbance

Background:

Chromatography:

The process of chromatography involves using the chemical differences between solutes in a solution to separate the various solutes for discovery or analysis. Just as we can separate the particles of sand and salt in a water solution by filtration and evaporation, we can employ chromatography to separate solutes of similar solubility from each other in a solution.

In paper chromatography, we rely on the differences in solubility of the solutes in the solvent. A dried spot of solution is applied toward the base of a strip of chromatography paper. The tip of the paper is allowed to contact a pool of solvent in the bottom of a chromatography chamber, or testtube, as the paper wicks the solvent up the length of the paper it passes through the dried solute spot dissolving the various solutes and carrying them up the strip of paper. Those solutes that are most soluble will travel farthest and fastest and the others will trail behind in order of solubility. If needed a comparison of the solutes

rates of movement along the paper can be determined using the distance traveled by the leading line of solvent as a reference.

The solutes, plant pigments in this case, that are separated will leave distinct lines of color where they are deposited. These can now be isolated for further investigation by spectrophotometry.

Spectrophotometry:

A spectrophotometer consists of an electromagnetic radiation source that can be set to emit radiation in range of wavelengths generally found to span most of the visible spectrum (approximately 350nm to 700nm). Once the desired wavelength is set the light is passed through a sample solution contained in a small specially designed testtube (approximately 13mm x 100mm). As the light passes through the sample (approximately 1mm thick) some of the light is absorbed by the sample and the remaining light passes on through and strikes a detector. The intensity of the light striking the detector is then transferred to an analog read out dial, or displayed digitally on the front of the spectrophotometer.

In order to accurately describe the amount of light actually absorbed by the chemical in question it is necessary to account for the background Absorbance by the remaining portion of the solution that you are not interested in. To do this you must prepare a solution without the chemical in it that can be used to adjust the reading of the meter to show a reading of 100 percent transmittance of light. It is also necessary to set the Absorbance of the meter to zero before starting. With the spectrophotometer "zeroed" you can now insert the sample and read the meter and determine the comparative transmittance (or Absorbance) of light by your sample. It is important to note that the meter looks very different in terms of transmittance and Absorbance, this is because the Absorbance represents an anti logarithmic function of transmittance: $A = -\log T$.

A spectral analysis is often used to show the Absorbance characteristics of a compound across the visible spectrum of light. In this type of analysis, the materials' Absorbance measured at even intervals from an initial wavelength to an ending wavelength. The resulting spectral analysis can then be used for comparison or identification of the material in question.

Procedure:

Unique Materials and Chemicals:

1. Paper Chromatography Chamber
2. Chromatography Paper, Whatman #2
3. Chromatography Solvent
4. Fresh Leaf Tissue
5. Spectrophotometer
6. Testtubes 13mm x 100mm
7. KimWipes

Procedure:

1. Determine and gather your materials
2. Determine the procedure that you will follow to extract the pigments from leaf sample
3. Determine a procedure for isolating the pigments into separate containers
4. Consult with your instructor and receive clearance to proceed
5. Perform a spectral analysis on each of your samples starting at 350nm and proceeding at 15nm intervals and stopping at 700nm
6. Prepare tables and record your observations
7. Graph your results of your spectral analysis for each of the pigments identified in your paper chromatograph

Data and Results:

Diagram and label the results of your paper chromatograph, be sure to identify each of the pigments you separated from your leaf sample.

Include your data tables of your spectral analysis along with your resulting graph.

Conclusion:

Discuss whether you met your objectives.

Discuss the method(s) of determining the identity of the pigments that you separated from your sample.

Discuss any problems or pitfalls you may encountered during any portion of the lab, include any changes you would make if you were to repeat your procedures.

Instructor Notes:

General flow of the lab should proceed as follows:

a) prepare the paper chromatography chamber

b) get a heavily concentrated sample of pigment on the paper

c) run the paper chromatograph and use scissors to cut each band of pigment from the paper

d) using forceps, suspend each paper in a 13mm x 100mm testtube containing 3mL of chromatography solvent until the pigment is redissolved in the solvent

e) analyze each pigment using a spectrophotometer

f) prepare a spectrograph of the results and research plant pigments to identify each sample

It will be important that you guide your students through the initial stages of this lab so that they are successful in separating and isolating the pigments from their paper chromatograph. Once a few groups figure out that they can cut

the band of pigment out of the paper and redissolve it in the chromatography solvent, you should probably address the entire class and make sure that all of the groups are following a similar procedure. Depending on the plants that your students choose, they will have different pigments that show up in their paper chromatographs, this will provide variety in the results obtained and make for interesting class comparisons of. If some groups are unsuccessful it may be necessary to combine the efforts of the two groups. This may also be necessary if you have access to only two or three spectrophotometers.

A problem that may also be encountered is not having a high enough concentration of pigment in the sample that will be used in the spectrophotometer. One solution to this is to run two or more paper chromatographs of the same sample at the same time and dissolve all of the pigment stained cuttings in the
same 13mm x 100mm testtube of 3mL of chromatography solvent.

Time Requirements:

Paper Chromatography: 1.0 period

Spectral Analysis: 1.5 periods

Appendix H

in vitro Pollen Tube Germination

Objectives:

1. The student will be responsible for the growth and maintenance of a Rapid Cycling Brassica (RCB) plant
2. The student will learn the life cycle of their plant
3. The student will identify major anatomical features of a plant
4. The student will prepare a solution to simulate the chemical environment of the RCB stigma
5. The student will collect pollen and initiate pollen tube growth observable with a microscope
6. The student will demonstrate proper staining and microscopic technique to observe sperm nuclei

Background:

We will use a cultivar from the Cruciferae family of plants that includes broccoli, cabbage, mustard and rutabaga, to name just a few. The plant we will employ is named *Brassica rapa* (*B. rapa*) after its ability to reach maturity in a relatively short time. *B.rapa* was developed in the 1980's under the direction of Dr. Paul Williams (et.al) at the University of Wisconsin-Madison. It was genetically selected and bred over a number of years to produce flowers and mature seeds in shorter and shorter cycles. The result is a plant we today call the Wisconsin Fast Plant, or Rapid Cycling *Brassica rapa*, often called RCB's or Fast Plants for short. The RCB's are useful because we can readily grow them in the lab, and they run a full cycle from seed to seed in just 36-40 days. The activity that you will be performing originally used a relative of *B.rapa* called B.

oleracea, however, the procedure transfers well to *B. rapa*.

Most high school students are able to identify the processes involved in the pollination of flowers (pollen is blown or carried from lower to flower). Some of those are able to tell what actually occurs during pollination (transfer of genetic information via the pollen). But very few can discuss what is actually being transferred, and how the transfer process is completed.

The goal of this activity, aside from the other stated objectives, is to initiate the growth of a pollen tube and witness its development in the laboratory under the microscope. Thus, this is called an *in vitro* process as opposed to an *in vivo* process.

The flowers of the angiosperms contain both the male and female reproductive tissue. The transfer of haploid genetic material is achieved by the pollen grain of one plant carrying its chromosomes to the pistil of another plant. Any number of plant pollen grains may make this trip but only the species compatible ones are able to produce a pollen tube that allows for the delivery of the sperm nuclei to the awaiting ovum so that seed development can proceed.

The development of the pollen tube is dependent upon a number of factors, one of those being the chemical environment of the flower's stigma. If these conditions are known then attempts can be made to replicate them in the lab. Analysis of the stigma's of RCB's has been shown to be concentrated with sugar and boric acid along with a variety of salts. The pH of the stigma has also been shown to be a factor in successfully germinating pollen tubes *in vitro*.

Procedure:

Unique Materials and Chemicals:

1. mature RCB pollen
2. electronic pH meter
3. Pasteur pipettes (or a small eye dropper)

4. toothpick
5. deep well depression slide with cover glass
6. methylene blue or aceto orcein stain (for staining DNA)
7. Chemical solutions:
    a) sucrose 0.585 M
    b) calcium nitrate 2.54 mM
    c) boric acid 1.62 mM
    d) hydrated magnesium sulfate 0.88 mM
    e) ammonium hydroxide 3.5 mM (aqueous from 30% stock)

Procedure:

Preparation of Media:

1. Measure the amount of each chemical required to prepare the correct molar concentrations of the final solution
2. combine these chemicals and dilute to 100mL with distilled water, confirm the pH to be between 8.5 and 8.8
3. Use the ammonium hydroxide (20-22uL) to adjust the pH if necessary, label and parafilm

Pollen Collection and Hanging Suspension:

1. Select a mature flower and use forceps to carefully remove one stamen, brush the anther across the center of a glass coverslip to remove some of the pollen grains
2. Use a clean Pasteur pipette to transfer a drop of your media solution to the coverslip and mix with a toothpick
3. Quickly and carefully invert the coverslip so the drop of media, containing pollen, is upside and hanging
4. Lower the coverslip over center of the deep well slide to complete the hanging suspension

5. Place the slide on your microscope stage and locate the pollen on 100X, view and observe the pollen at 400X
6. Note the time and record as time zero, observe and record the appearance of the pollen grains in a data table
7. Record your observations every 10 minutes
8. Once the tubes begin to appear, describe the length of tube in Pollen Grain Units (PGU's), where one PGU = the diameter one pollen grain
9. Choose several tubes to continue observing and recording data, select one of these to sketch for your data and results

Preparation of Stain and Squash:

After the pollen tubes have discontinued their growth you can now attempt a squash and stain for the presence of DNA in the tube or pollen itself. DNA will absorb and bind both methylene blue and aceto orcein stain, you may use either.

1. Place a drop of your stain on a clean microscope slide
2. Carefully remove the coverslip from your hanging suspension and place it on the drop of stain, allow two minutes for the stain to be absorbed by any DNA
3. Proceed by using a folded paper towel and your thumb to firmly press down on the coverslip, this should crush the structures likely to contain any chromosomes and allow them to be viewed, view at 400X or for even better viewing, 1000X if oil immersion is available
4. Look for areas that have absorbed more stain than the surrounding crushed structures, these are likely to be the chromosomes containing DNA

Data and Results:

Include all of your calculations for the preparation of the chemical media,

as well as, your data tables of your observations. Include a sketch of one of your pollen grains with its pollen tube.

Conclusion:

1. Prepare a labelled drawing of typical flower with its reproductive anatomy.
2. Discuss the difference between *in vitro* and *in vivo*.
3. Why do you think the media prepared was so concentrated with sucrose?
4. The binucleate pollen grain of RCB's carries genetic information to be delivered to the plants haploid ova. The trace the events that occur in the fertilization process.
5. Studies have shown there is a degree of incompatibility between a pollen grain and the stigma of the same plant. Pollen tube growth tends to be inhibited when self pollination occurs. What can you conclude about the evolution of plants from these findings?

Instructors Notes:

The Wisconsin Fast Plant is a versatile lab plant that can be utilized in a number of different areas in your science scope and sequence. The *in vitro* pollen tube activity described here is best suited for inclusion during units on plant evolution or reproduction. However, it can inserted during a unit on genetics or plant anatomy if that is when you happen to be growing plants. If you are not growing plants at the time that you wish to perform this lab, then you need to plant seeds about 17 days before you wish to collect pollen for use.

The lab is targeted for advanced students due the chemistry involved in the preparation of the simulated media. It is easily adaptable to introductory biology, although, the instructor will need to prepare the media in advance for the students.

The stigma of RCB's presents a specific chemical environment for the germination of a pollen tube by a pollen grain, as was determined by thin layer chromatography (Hodgkin and Lyon 1982). *In vitro* replication of these conditions is successful in a slightly alkaline environment (pH of 8.5 to 8.8) controlled by using ammonium hydroxide solution (30% stock concentrated NH_4OH, final concentration of 3.5 mM). Other ammonium salts, such as, ammonium chloride and ammonium nitrate have not been successful in stimulating pollen tube germination. The amount of ammonium hydroxide needed will vary depending on the pH of the distilled water used in the preparation of the media. For media that had an initial pH of 5.8, 22uL of NH_4OH was required to adjust the solution, when the pH was 7 only 20uL of NH_4OH was needed. It will be important to have your students check the pH of their solution prior to adjustment of pH and proceed by 5uL increments until the desired pH is reached.

It is important that the media be prepared no more than 24 hours in advance, any longer than this and its ability to initiate pollen tube growth is diminished. Under ideal conditions the growth of a pollen tube should begin within fifteen to twenty minutes after immersion of the pollen grain in the media. Tube growth should continue for another 90-120 minutes. It may be necessary for students to return during the day to observe their suspensions. A suggested time line would be:

Day one: student preparation of media, store cold

Day two: recheck pH and preparation of hanging suspension, allow media to adjust to ambient temperature

Day three: staining and viewing of nuclear material

The staining of the pollen tube for sperm nuclei is a time sensitive issue. If the pollen tubes are allowed to set over night, the concentrated sugar solution

may begin to crystallize. This may be best accomplished by having the students who are interested in the stain and squash returning later in the day, or after school to complete this part of the activity.

In order to adequately view a squash for genetic material, an advanced microscope with oil magnification of 1000X is recommended. There may not be a high incidence of success in staining and identifying nuclear material, and there is some question as to the success of sperm nuclei migration under *in vitro* conditions. The result of the squash may be a mass of genetic material at the site of the pollen grain, instead of down the pollen tube. Both methylene blue and aceto orcein stains have been shown to be effective in staining DNA under these conditions.

Time Requirements:

Media preparation: 1.0 period

Pollen tube germination: 1.0 period

Chromosome squash: 1.0 period

Appendix I

Growing Wisconsin Fast Plants

The best source of information for the use of Fast Plants (RCB's) is Carolina Science and Math, the authorized distributor of seeds and materials relating to Wisconsin Fast Plants. Most high schools have copies of the most recent catalogs from Carolina Science and Math, or the company can be contacted directly at the following address or phone:

Carolina Science and Math

Burlington N.C. 27215

ph: 1(800)334-5551

Basic materials for growth can be purchased from Carolina, or constructed by the instructor. It is advisable for starters to purchase equipment and familiarize themselves with the materials and procedures, and, if you are so inclined, you can adapt or build new materials to suit your own instructional needs.

Basic supplies:

* Basic Wisconsin Fast Plant Kit
* Light Bank, 40 Watt Fluorescent bulbs
* Light Bank Stand
* Brassica rapa seeds

The daily growth cycle for the Rapid Cycling Brassica's is highlighted in Figure 3 below:

Figure 3 - Life Cycle of Rapid Cycling Brassicas

Days 1-3 --> germination, cotyledon leaves appear (day 3)

Days 4-9 --> true leaves (day 5), flower buds develop (day 9)

Figure 3 (cont'd)

Days 10-12 --> stems elongate and buds enlarge

Days 13-17 --> buds open, pollination may begin, pollinate on days 13,15,17

Days 18-22 --> petals drop, seed pods elongate and swell embryonic development begins

Days 23-36 --> embryos develop and seeds are formed, ovary becomes a silique (pod) and will dry, remove from water

Days 37-40 --> dry down and harvest seeds on day 40

The RCB's were selected for their rapid growth potential and grow most rapidly when they are exposed to the light source twenty four hours per day. The light bank should be positioned so that the bulbs are approximately 5cm above the plants. This distance will become askew for some of your plants as they begin to develop and grow to non uniform height.

One trouble with the all day all night light growing conditions is that it promotes the grow of algae that will compete with your plants for nutrients and cause rotting of the thin stem tissue. To counter act this Dr. Williams suggests using copper sulfate soaked discs that are included with the basic RCB growth kit available from Carolina Biological. These algae inhibitors, as well as other supplies, can be purchased as needed from Carolina Biological.

The use Wisconsin Fast Plants are almost guaranteed to be successful in the classroom for students at all levels.

Appendix J

Suggested Test Items For Lab Activities

Multiple choice test questions are readily available for use by textbook companies that supply text materials for a district. These are generally good questions and mirror the quality of questions desired for advanced level classes. The following are test problems and short answer essay questions that can be used as seen fit by the instructor.

Solutions and Buffers:

1. Show how you would calculate the number of grams required to have 3 moles of glucose ($C_6H_{12}O_6$).

2. Show how you would prepare 500mL of a 0.1 M solution of acetic acid ($C_2H_4O_2$).

3. If you used 22.5 grams of glucose in preparing 500mL of solution, what would be the final molarity of the solution? Support your answer by showing all of your work.

4. Prepare a graph showing the effective buffering range for a generic buffer system. Label the graph with the range of the buffer, the pK, and the fraction of base and acid at the pK, and the upper and lower ends of the buffering range.

5. Describe the Henderson-Hasselbach equation and discuss the relationship between the acid-base ratio, the pH and the capacity of a buffer.

6. Why is it important that living systems are "buffered"?

Chromatography:

1. Discuss the differences between paper and column chromatography.

2. In attempts to separate a sample of brown food coloring collected from

a cafeteria food product you run 2mL elutions of Isopropanol at the following concentrations netting the results below:

solvent conc.	eluent collected
70%	clear
60%	clear
50%	light brown
40%	brown
30%	brown

Discuss how and why you would proceed from here to separate the brown food sample into its constituent dyes.

Spectrophotometry:

1. In the Beer-Lambert equation A = elC, explain why the Absorbance is directly proportional to both the concentration and the light path width .

2. Why is important to "zero" the spectrophotometer and use a blank for setting transmittance prior to measuring a sample?

3. If you were given a sample of protein of unknown concentration to analyze describe how you would treat the sample to determine the concentration of protein present.

Plant Tissue Culturing:

1. During a lab studying the effects of hormone inhibitors on plant shoot and root development, you mistakenly get your test samples confused so that you do not know which plant has the root hormone inhibitor and which has the shoot hormone inhibitor. You decide to continue, hoping that your instructor overlooks the missing labels. What will you look for, as the plants develop, to get the correct hormone inhibitor label on the correct test plant? What hormones are likely to be inhibited in each test plant?

2. Tissue culturing must be performed under aseptic conditions. What

does this mean and what are some steps that are carried out to ensure that these conditions are met?

Seed Germination and Environmental Impact:

1. What is likely to be the first anatomical feature, of a plant, that you would observe as it germinates?

2. Describe how you could set up a controlled experiment to test for the impact of an environmental condition on the growth of an organism.

Plant Pigments: Chromatography and Spectral Analysis:

1. The leaves of some plants appear only purple in color, what would expect to find if you were to run a chromatograph on an extract from one of its leaves.

2. How would the spectrograph of the plant described in question number 1 differ from a spectrograph of green spinach leaves? Show a graph that approximates this difference.

3. During a botany expedition you discover a plant that has only white leaves, stems and flowers. Intrigued, you perform a spectral analysis on an extract of the leaves and the only spike on the graph occurs between 295nm and 330nm. What is your best explanation of these results?

***in vitro* Pollen Tube Germination:**

1. Why do think plants evolved the process of pollen tube germination and growth?

2. Pollen grains have found to be mononucleate, binucleate and even trinucleate. What does this mean, and does it impact the fertilization process of the plant ova?

3. From your knowledge of pollen tube germination, how do you think a plant is able to prevent self fertilization of its ova by its own pollen?

APPENDIX K

TEXT OF EXIT SURVEYS

SURVEY PART 1: BIOTECHNOLOGY

Please rank the following items based on your confidence level in working with the equipment or techniques described. Consider your confidence level both before and after taking AP Biology, use the scaled key shown below.

zero confidence (1)	little confidence (2)	fair confidence (3)	good confidence (4)	excellent confidence (5)

BEFORE AFTER

_____ _____ 1. Ability to properly use a spectrophotometer (Spec 20).

_____ _____ 2. Ability to convert information from % Transmittance or Absorbance to graphical information for analysis.

_____ _____ 3. Ability to properly use a micropipet.

_____ _____ 4. Ability to accurately transfer specific volumes of solutions using a standard pipet.

_____ _____ 5. Ability to make a solution of specified concentration from stock chemicals.

_____ _____ 6. Ability to use serial dilution technique to acquire a diluted solution from a stock solution.

_____ _____ 7. Understand the purpose and function of an autoclave.

_____ _____ 8. Ability to produce a formal lab write up to design, run and analyze an experimental procedure.

_____ _____ 9. Utilize aseptic technique in the laboratory.

_____ _____ 10. Understand the use Tissue Culture Media in the laboratory.

_____ _____ 11. Ability to prepare a Fast Plant Mini Growth Chamber.

_____ _____ 12. Understand the purpose and preparation of a pH buffered solution.

_____ _____ 13. Ability to apply paper chromatography as a separation technique.

_____ _____ 14. Ability to apply column chromatography as a separation technique.

_____ _____ 15. Ability to use spectral analysis to to distinguish plant pigments.

SURVEY PART 2: BIOTECHNOLOGY

Please rank the following items from 1 to 5 based on the Key below :

Strongly Disagree (1)	Disagree (2)	Neutral (3)	Agree (4)	Strongly Agree (5)

_____ 1. I have a better understanding what biotechnology is now than before I completed AP Biology.

_____ 2. I believe that as a citizen I am able to view biotechnology research more objectively than before completing AP Biology.

_____ 3. When I view or read news reports concerning biotechnology, I have a greater understanding of their content now than before completing AP Biology.

_____ 4. I believe that I am better prepared to decide on a field of study in post secondary education after completing AP Biology.

_____ 5. As a result of completing AP Biology, I will be more likely to enroll in science courses in my post secondary education.

_____ 6. I am less skeptical of Industrial Biotechnology now than I was before completing AP Biology.

_____ 7. I am less skeptical of Medical Biotechnology now than I was before completing AP Biology.

_____ 8. I believe that I am more competent in a laboratory than I was before completing AP Biology.

_____ 9. As a result completing AP Biology, I believe that I am better prepared to succeed in post secondary education.

_____ 10. I believe that plants can utilized as an interesting method for studying biology concepts.

Appendix L

Construction of Mini Growth Chamber

Figure 4 - Top View of Chamber

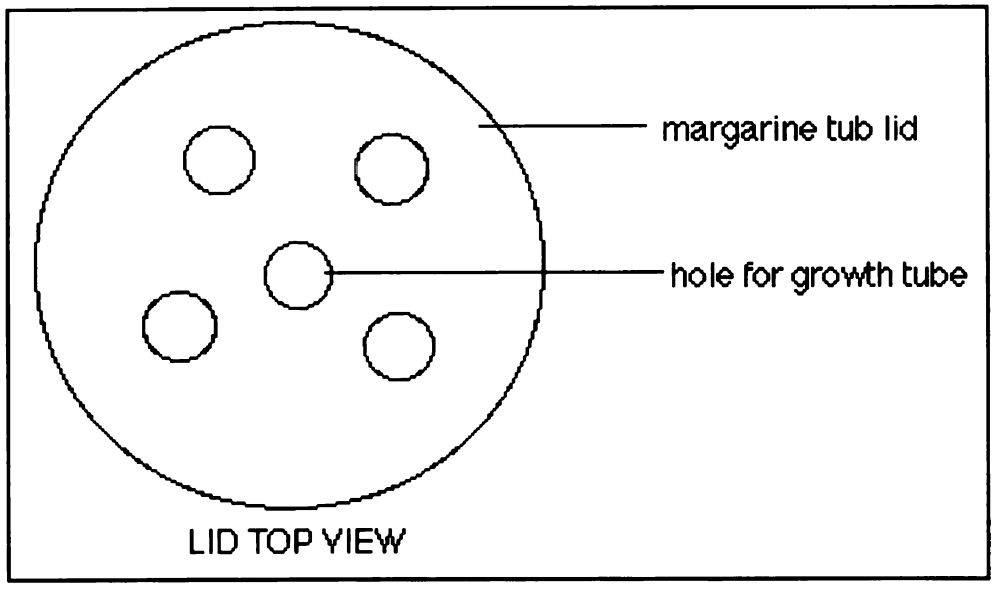
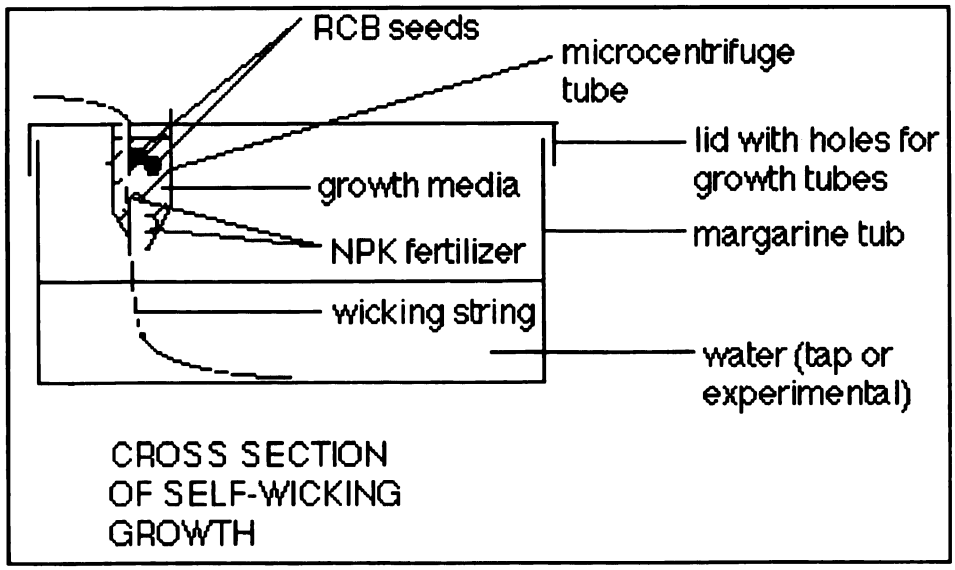


Figure 5 - Cutaway Side View of Chamber



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