HIGH SCHOOL MOLECULAR BIOLOGY UNIT FOR ADVANCED BIOLOGY STUDENTS

By

Bruce Lee Buysse

A THESIS

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

MASTER OF SCIENCE

College of Natural Science

1992

ABSTRACT

HIGH SCHOOL MOLECULAR BIOLOGY UNIT FOR ADVANCED BIOLOGY STUDENTS

By

Bruce Lee Buysse

The purpose of the high school molecular biology unit was to teach the basic principals of protein synthesis and recombinant DNA to advanced biology students through the use of hands-on lab experiments. The students used school made equipment to do five experiments using recombinant DNA techniques. This lab approach to teaching replaced a traditional lecture-discussion approach. The students were interviewed and tested prior to and at the conclusion of the unit

The core of the unit was five integrated lab exercises. The labs started with DNA isolation and properties, then continued on with bacterial transformation by plasmids and concluded with plasmid isolations evidenced by gel electrophoresis.

The findings of the study were that the unit solidified the learning of the previous biology courses after hands-on lab experiments with recombinant DNA. The students were also very excited about performing the labs and learning from them.

I want to dedicate this thesis

to my children

April, Sarah, Josh, and Emily

and especially to my wife

ELLEN

their understanding, patience and encouragement

allowed me the time and gave me the inspiration to

complete this research

ACKNOWLEDGEMENTS

I would like to thank the following people for their assistance in the completion of this study:

Dr. Merle Heidemann

Dr. Clarence Suelter

Dr. Howard Hagerman

Dr. Steven Triezenberg

Dr. Marty Hetherington

Mr. David Devore

Mrs. Helen Waldo

my family

April, Sarah, Josh, & Emily

Buysse

most especially

my wife, Ellen

TABLE OF CONTENTS

VI. Appendix

INTRODUCTION

Overview:

Science, unlike most other school subjects, is growing with the addition of information discovered yearly. Biology textbooks and courses get larger and more involved every year. I am overwhelmed with the idea of trying to get a representation of all the new in formation to my students. The approach I used for many years was a "shot gun" approach. I covered a little in all areas of the science curriculum but did not spend a great deal of time with any one area in the Advance Placement Biology class. I felt I was in a foot race with the clock. I did not spend time looking around at the scenery on my journey through Biology. I could only see the finish line. Often I left class physically out of breath and tired from trying to cover so much material in so little time.

I taught the Molecular Biology unit this same way. I covered the basic principles and discussed the advances made in biotechnology but did not spend time in class discussion and did no lab work to reinforce molecular biology. I felt successful with the unit in the short term goal of preparing students to pass the chapter test, but felt a failure in the long term when students had a difficult time applying the material discussed in class to other biological concepts like human genetics, evolution, and classification. Semester exams and national achievement tests also showed that the students lacked of the ability to apply knowledge learned in class.

I consoled myself by believing that I was doing the best I could, my students still liked the course and I could see no other way to do it. My background in biotechnology was minimal because it was not part of my undergraduate studies twenty years ago. Not having a background in biotechnology also made it difficult for me to feel comfortable reaching it or doing labs in this field.

Working with recombinant DNA for the last three summers, my "comfort" level for the material rose considerably. The summers of preparation gave me the opportunity to work with the labs directly and learn how to do them. The equipment to do the labs was very expensive when purchased through a supply company. The summer work allowed me to develop low cost equipment such as 24 volt power supply, micropipettes using syringes, electrophoresis chambers from food storage containers, and dye chambers for the gels.

I could see that a complete revision was needed to teach the course because my old lecture technique for teaching would be ineffective. The labs not only reinforced the material but also enriched it and challenged the students to apply it to future problems. The labs offered the students an opportunity to do work in an area they had not experienced before, with equipment they had never used. I hoped that through this experience some of the students would select this area of study to do an extensive project during the second semester or to look into study beyond high school.

The old molecular biology unit was about two weeks in length. The total time of instruction was ten 55 minute periods from beginning to the end of the molecular biology unit. The new lab oriented revisions increased the molecular biology unit to about five weeks or twenty-five 55 minute class periods. The increase in the amount of time spent

on the unit meant that other units had to be revised and revamped. The implementation of the unit not only improved the molecular biology unit but also led to the revision of the following units which include evolution, animal and plant taxonomy and the introduction to plant physiology. I concentrated these units and combined these separate labs into one multi-part lab that seemed to tie the concepts together better.

One of the largest changes in the new approach to molecular biology in comparison to the old lecture approach was extensive use of lab periods. Prior to the labs, I discussed with the students how it was done through overlays, chalkboard, and demonstrations. The new approach followed a lecture-discussion in the classroom concluded by one or two day lab experience which demonstrated the principle and future possibilities of the technique. These labs all had the potential possibilities of being expanded by the students for further study. The newness of the labs and the equipment required more prep time for me and more time in class explaining how it all worked. At the conclusion of the lab, a portion of the final lab day was used to discuss the results and have a group discussion about what the data meant and how to interpret it.

I found myself much more excited in teaching this unit I think my excitement was passed on to the students because they had more questions and discussion seemed generated in classroom more easily. All class members asked questions and together we tried to answer them. Time seemed to pass quickly and all the students got involved with more and more aspects of the labs. The five extensive labs had to be written up in a lab report by the students and submitted to me. After am initial phase of complaining, most seemed to write the labs with good detail. The first two labs, DNA isolation and properties, were written up simply. However, the final lab on DNA recombination was

written very completely and showed time and effort by the students.

Written Material/Scientific Literature:

There is abundant scientific literature in the field of molecular biology. Through my reading I concluded that this literature fell into two major categories. The first and major category was found in scientific journals about research dealing with specific bacteria, techniques, problems, and discoveries. The second category of material was written to help explain these research concepts and how they relate to each other and fit into a cohesive picture of molecular biology. These articles appearing in scientific periodicals and teaching journals formed the core of my reading for thesis research.

As I read through the literature, I became aware of the scientific information needed to teach this topic and also discovered ideas about presenting molecular biology concepts to high school student. American Biology Teacher, Biotechnology Education, and Science Teacher presented a wealth of information and ideas that I included into my research and thesis.

In reading the literature, I was surprised at the lack of understanding of DNA and concepts of biotechnology among the American public. According to Linda Dixon(1988) " as of 1986 57% of American said that they had little to no understanding of DNA". She states that in 1987 a majority of Americans had not ever heard the word "biotechnology".

This lack of knowledge of molecular biology makes the task of instruction a challenge to a teacher. Not only is it important to teach concepts of molecular biology

but it is also important that the students understand these principle and how this technology will affect their lives now and in the future.

The purpose of an introductory molecular biology unit in high school is to provide students with the knowledge of the structure and function of DNA so that they will be informed citizens and be able to make educated decisions as adults about the products of biotechnology. I wanted my students to appreciate the application of molecular biology to their lives.

Ralph Lewis(1988) points out an important concept for teaching molecular biology or any science to high school students. He concludes that to improve education at the high school levels, teachers need to change descriptive biology from lectures to hypothetic-deductive biology. Hypothetic-deductive biology is the teaching of abstract concepts to students and expecting them to use deductive logic to tie together concepts. He states that a survey of the advancing fronts of biological knowledge in molecular biology shows that most biology today is highly theoretical. Because it is theoretical, it is hard for the student to understand and envision uses for it. Theoretical science does not mean it is impractical to use in a classroom, but it needs to be reinforced with techniques that will help students visualize its implications in the world of science.

Robert G. Thompson(1988) sums up the need to move students from the theoretical to the practical aspects of molecular biology by using the term Technology literacy. He defines this term as the ability of students to have a foundation of skills that will prepare them as citizens to make sound evaluations of biotechnological decision which involve environmental issues, safety concerns and ethical issues. He paraphrases an old Chinese proverb:

"Tell me and I will forget show me, I might remember Involve me and I will understand"

This old Chinese proverb sums up the whole idea of teaching high school students. As an educator, I try to present as much scientific knowledge to my students as possible. The science of biology increases its wealth of information yearly. Each year there is more and more to teach and seemingly less and less time in which to do it. For years, I have lectured daily attempting to fill the students minds with all the concepts I thought they needed. The task is overwhelming and basically impossible to accomplish. As the years have gone by, the hand-on aspect of biology, labs and research projects have been done less and often replaced by demonstration and visual aids.

The Chinese proverb says that unless you involve me, I will not understand. In teaching the molecular biology unit, I attempted to step away from lecturing to students and towards the idea of students discovery of concepts so they would understand the principles.

Dr. James D. Watson(1989) points out the need for an understanding of molecular biology by all citizens. DNA research is beginning to have medical and ethical consequences that will affect our daily lives. This research will help us plan for the future of our children and they for their children's future. Watson states that we must realize that we are living in the midst of a virtual biological global rush, searching in areas where no one has traveled before. He continues on to say that we need also to train a vast number of scientists who want to work with recombinant DNA procedures focusing on cures for genetic diseases.

The challenge of teaching these concepts of recombination DNA technology to

high school students in a way that they can see and understand is a challenge at the secondary level. Linda Dixon(1988) notes that despite its mystical and seeming magic, recombinant DNA technology is a straight forward set of procedures which can be been performed by teenagers. She believes that recombinant DNA technology is critical to development of a complete lab program that students can do and understand.

The development of a complete lab program to supplement the molecular biology unit I was to teach was the program that I undertook in my thesis. I wanted students to be familiar with the terminology of the discipline and then to use lab experiences to help them understand the concepts. I think that only through this combined effort could I leave students with concepts they could take out of school and use in their lives as adults.

In preparing the written material used in the molecular biology unit, I found that the textbook Biology, Journey into Life by Arms and Camp (1990) had a very good but brief approach to molecular biology. High school texts that completely cover the material, yet are not too detailed so the students won't read them, are difficult to find. Arms and Camp have done a good job keeping the unit on molecular biology brief but complete enough to provide a foundation for understanding. However, I could see that supplementary material was required to adequately explain the concepts in lecture so the students could not only understand the labs but be able to see their applications into the field of biotechnology.

As I began to teach the unit, I found it necessary to use a series of overlays(Appendix B) to help clarify the concepts of DNA structure, protein synthesis especially translation and transcription, operon theory, and bacterial transformation.

The protein synthesis chapter(10) of Arms and Camp required the use of special

audiovisual material, and felt board, to help the students visualize protein synthesis. This board uses pieces of felt to illustrate the concepts of DNA, T-RNA, M-RNA, ribosomes, proteins, amino acids and their roles in the process of synthesis.

I read a variety of sources to help supplement the Arm and Camp text. Most of the information came from Scientific American, Science Teacher, Science, and American Biology Teacher articles that were very detailed in more specific areas of research but did not help explain the basic concepts I needed to teach. I reviewed the textbooks that I had used in teaching college introductory courses such as:Biochemistry by Champe and Harvey(1987) and Biochemistry by Stryer(1988). Although each of the texts provided me with bits and pieces of information to supplement the text, none of them gave a complete overview or simple outline to follow. I found the best text that helped me in preparation of my lecture notes for molecular biology was the book: DNA Science: A First Course of Recombinant DNA Technology by David A. Mickels and Greg A. Froyer(1990). This text was written with the advanced high school student in mind. The book was divided into three parts. Each of the first eight chapters discussed a basic theme of molecular biology and was detailed enough for high school students but not beyond their range of experience. These chapters were extremely enlightening; for the unit. This book began with the historical picture of the people involved in research. The book told a very human story and helped me humanize molecular biology. In following chapters, the basic tools and techniques of DNA science were explained. The chapters started with the basic terminology such as recombinant DNA, Plasmids, restriction enzymes, and bacterial transformation. The later chapters of the first part of the book discussed the genome project, DNA probes, and constructing a DNA library, cancer

research and applying DNA to Human Genetics. These chapters provided the information I needed that was not found in the student's text. The book was written clearly and simply so that I could apply it directly to my class presentations. I was able to answer many of the questions the students asked because of what I learned from this text.

The second part of DNA Science: First Course consisted of recombinant DNA laboratory experiments. The labs were written very clearly and were easy for me and my students to understand. I primarily used the labs that I developed in summer research and I used the labs from this book to help trouble shoot any problems I might encounter. It was also a consolation to see that the labs I had written were very similar to the more polished labs presented in the text. The additional labs in the text could be done by students with interest in further work. These labs also used materials that would be readily available to a high school teacher. Each lab had a section at the end called "for further research" which made the labs very open ended.

The final section of the book, DNA Science: First Course contained an extensive appendix which included equipment needed and where to locate it, recipes for media, reagent, stock solutions, restriction maps of DNA and plasmids.

The book overall was the best source as I prepared the molecular biology unit, and it was very readable and understandable for me and my students.

Outline of New Labs:

The molecular biology unit followed units on the cell and cellular processes. This new unit was included in the Cell Biology unit and bridged to the next unit on Genetics. The molecular biology unit was needed to familiarize the students with the general concepts such as chromosome, gene, DNA structure and properties, DNA replication and mutations. From these beginnings, I moved into discussions of bacterial transformation, bacteriophages, protein synthesis, and the operon theory of gene expression. Student understanding of these concepts leads into the application of molecular technology including genetic engineering, restriction enzymes, cloning and gel electrophoresis.

In order to start the unit on a solid foundation, I presented the basics of molecular biology: DNA in a lab/lecture format.

Although DNA structure and function had been introduced earlier in the Biochemistry unit, I reviewed basic nucleic acids structures, the base-pair combinations, and the history of nucleic acid research.

This review was followed by an explanation of protein synthesis. The discussion began with an overview of the protein synthesis. The discussion began with an overview of the protein synthesis process and was followed by a detailed explanation of transcription, transformation, the enzymes involved and codons. The discussion focused on the "Operon Theory" and how it related to cancer. An historical picture was given of the researchers who worked on the mechanism of protein synthesis especially Beadle

and Tatum.

Next was the discussion on the application of the DNA knowledge to technology, emphasizing plasmids, restriction enzymes, gel electrophoresis, cloning, bacterial transformation and restriction mapping. These concepts led into a discussion of the present uses and future potentials of DNA technology. Cancer research as related to DNA technology was discussed extensively in class on numerous occasions.

The main objective of the thesis was to present materials and activities to students so that they would understand the research in molecular biology and allow them to experience the lab techniques used in that discipline. Molecular Biology is a recent addition to most textbooks and courses in high school. Students have a basic understanding of the cell but seem to have a hard time grasping the function of nuclear DNA. I have long been aware of the difficulty in teaching these abstract but essential concepts to students. The techniques of recombinant DNA have become available to high schools in recent years, so I decided to use these techniques in a series of labs to help the students visualize concepts. These concepts include bacterial transformation, gene slicing, plasmid cloning and removal and action of restriction enzymes. I found that students who do hands-on labs, take data and visually see results have a better understanding of recombinant DNA technology.

The central theme that I wove through the six week unit was the versatility of DNA in the cell illustrated by a series of five multi-part labs(Appendix A). During discussion of the first chapter of the Molecular Biology unit, which was a review of DNA structure, The students isolated DNA form tissue and massed it on a glass stirring rod. The students were also able to look at cells and nuclei through a microscope. The

purpose of the lab was to have the students observe a quantitative mass of DNA on the glass rod.

The next lab demonstrated to the student the properties of DNA.(Appendix A) In this lab, the students obtained a quantitative mass of DNA and analyzed it with chemicals, temperature and enzymes.

The discussion of DNA properties proceeded into a discussion or DNA function, concentration on protein synthesis and the operon concept. This material was not reinforced by a lab at this time.

Most of the lab techniques were performed in the last section of the unit. First was a discussion of recombinant DNA terminology. Students visualized DNA, the function of restriction enzymes and the plasmid's role in recombinant DNA with a "dry lab".(Appendix H) The dry lab was done with scissors and paper models of plasmids. The students constructed a DNA chain, plasmid DNA and worked with two known restriction enzymes EcoRI and BamHI. I wanted the students to learn how a transformed bacteria is created.

Experiment #3(Appendix A) was a transformation of bacterium E. coli using plasmid pUC 18 to confer ampicillin resistance. The students made the bacteria competent and then mixed the plasmid with the bacteria. The bacteria was cloned and them tested against an ampicillin plate. The students saw that those bacteria that were transformed could grow on the ampicillin whereas those not transformed did not grow on the plates. This lab stressed the importance of the genetic material(genes) of the plasmid and how it affected the bacterial survival. Also as part of this lab, transformed bacteria was induced to turn off one gene and turn on another. An inducer turned the media blue.

This very visible lab demonstrated the operon theory of gene expression by turning on another. The lab also allowed students to count colonies and estimate the efficiency of the transformation. The complete lab with all of its parts took most of one week. Each part helped reinforce the discussion in class of transformation and build on the previous lab portion.

The culture of transformed cells was used in lab #4(Appendix A) to try to remove the plasmid introduced into the bacteria in lab #3. Plasmid isolation is a difficult technique and a variety of procedures can be used. I divided the class into four groups and each group tried one of four techniques: 1)filtration, 2)boiling, 3)lysis, and 4)modified lysis.

These four techniques resulted in the students obtaining a tube of a 20 to 50 microliter sample of supposed plasmid. The samples of supposed plasmids obtained in lab #4 were used for the first part of lab #5(Appendix A). This lab would demonstrate the presence or absence of plasmids by gel electrophoresis. This, the final lab of the unit, was designed to bring together all the aspects discussed in the unit and to visualize and stimulate interest in other aspects of recombinant DNA work.

The final lab of the unit used gel electrophoresis to first isolate the supposed plasmid from lab #4 and then to continue with gel electrophoresis to separate DNA fragments which were digested by restriction enzymes.

The five multi-part labs gave the students the opportunity to perform a few basic hands-on activities with recombinant DNA. The first two labs made the DNA visible to the students and allowed them to see its properties. The last three labs gave the students the opportunity to work with a living organism, and DNA to it, demonstrate that it was

there and working, then remove it again and assay. The labs were and opportunity to use the technology described in class discussions in the lab. The labs also gave the students an opportunity to follow up each experiment with a lab report using technology from the chapter discussion in the lecture.

At the conclusion of each lab, a discussion was held to sample outcomes, discuss difficulties and challenge students with future problems. As the labs progressed, the discussion after each lab experience for more extensive by adding future applications and problems with genetic technology. Students discussion on biotechnology increases with each lab done. They were supporting their views with material that we had discussed in the chapter. Overall the labs really made the unit successful to me and my students. This success is supported by the post interview essay question at the end of the post test. Success is also measured in the enthusiasm I observed after twenty years of teaching high school.

INSTRUCTION

Basic Outline:

The instruction of the molecular biology unit was based on Biology: A Journey into Life by Arms and Camp(1989). Five experimental labs were done by students during this unit. The entire unit took about twenty five class periods of fifty minutes each. This unit included a pretest and post test of the unit and two test given during the unit. The first test followed Chapters 9 and 10 and the second test was over chapter 11. Preceding the unit of instruction, a series of interviews were conducted by me with the students to better understand their prior knowledge, scientific interest levels and feelings about science in the future. The post test contained the same questions as the pretest plus a question that asked them to discuss their learning form the labs and thoughts on the unit.

The unit began with a review of DNA that took about three days. This review involved a discussion about DNA including the discoveries that lead to our understanding of the molecule. Most students only knew of Gregor Mendel and Watson/Crick and research in genetics. They seemed to believe that these three men did all the work. I supplemented the DNA research with the work of Griffith(1928), Hershey & Chase(1952), Chargoff(1940), and McClintock(1940). I wanted these chapters to build a picture of the story of DNA research. A majority of the material was review from the

Biochemistry chapter earlier in the year. This chapter on DNA took four days to go through and during that time two labs were done.

The following is summary of he daily teaching of the molecular biology unit: Chapter 9-DNA Genetic Information:

Day 1: Pretest of 45 multiple Choice questions (Appendix D)

Day 2: DNA Terminology including DNA, chromosome, gene, replication, DNA polymerase, amino acid, protein, polymers, nucleotides; and explanation of Bacterial transformation experiment by Griffith(1928)

Day 3: Work of Hershey and Margaret Chase(1953) with bacteriophages, work of Franklin and Watson and Crick work in DNA structure

Day 4: Lab #1 DNA Isolation(Appendix A): The lab was to isolate nuclei from tissue, and remove DNA from the solution for study. The lab went well and the students were able to spool large amounts of visible DNA on glass rods. For most of the students this was the first time they had seen DNA other than as a concept in a book.

Day 5: Lab 2 Properties of DNA(Appendix A): This lab followed the procedures of Lab #1 initially and then experiment with the mass of DNA on the stirring rods as to its properties with heat, types of cooling, and effects from and enzyme: DNAase 1. All of the properties were demonstrated by the lab.

Day 6: We discussed DNA replication, DNA repair, the structure of

chromosomes, genes, and work of McClintock.

After this thorough review of DNA and its structure and properties, I moved into a discussion of the role of DNA in the cell: transcription and translation.

Day 7: The parameters of protein synthesis were discussed and defined. These included RNA, mRNA, tRNA, gene, transcription, translocation, and protein. A felt board with different colors and shapes(Appendix E) used to represent the DNA, mRNA, tRNA, ribosomes, amino acids, and protein helped students with the basic concepts of protein synthesis.

Day 8: A brief review of the felt board began the day followed by a discussion of how the code for the protein is formulated. The concepts of codons. A brief overview of the concept of mutations and was discussed, stressing the effects of a one base pair change. The major concept of the day in class discussion was of transcription of DNA code.

Day 9: An in depth discussion about the structure of mRNA and specificity of tRNA with the ammoniacal site and anticodon was held first. The second major concept of the day was translocation at the ribosome. The concept of translocation is a difficult one for the students to follow, so I used a variety of overlays (Appendix B) to help explain the process.

Day 10: Since this was the final day of discussion concerning protein synthesis. I focused the time to tie all the material into one

cohesive picture especially the "operon theory". I gave the students a handout (Appendix F) on the operon to fill in as we discussed the material. The terms included repressors, inducers, promoters, introns, and exons.

Day 11: Review of the material in Chapters 9 and 10

Day 12: Test over Chapter 9 and 10. The test consisted of 70 objective questions and two 4 point essays and one 10 point essay.(Appendix G)

Day 13: Review the test and begin chapter 11 New Genetics: Molecular Genetics with a discussion of the terms that will be introduced in the chapter. These terms include genetic engineering, hybridization, restriction enzymes, reverse transcriptase, clone, plasmid cloning, recombinant DNA, & oncogenes.

Day 14: We discussed how plasmids are formed, the action of restriction enzymes, and cloning of transformed DNA material.

Day 15: The students did a "dry lab"(Appendix H), in which they could see how restriction enzymes work by using paper models of DNA and plasmids they could cut. The students could visualize the concept of "sticky ends" and see how the plasmid can join with the DNA. All the students constructed models and some even made multiple cuts and a variety of DNA.

Day 16: I followed up this Dry Lab with Lab #3 Bacterial Transformation of E.coli with pUC 18 plasmid(Appendix A). First the students

make an E. coli competent. Secondly, they add pUC 18 plasmid to the colony creating a transformed E. coli colony. The transformed colony contains a gene for ampicillin resistance.

Day 17: The colonies from day 16 were counted and recorded. We then began work with the Lac Operon. The students took samples from the bacteria that grew on the plate(+) and transferred them to a plate of agar with X-gal(5-Bromo-4-chloro-3Indolyl b-d-Galactopyranoside) and IPTG (Isopropyl b-d-Thiogalactopyranoside). The media required the transformed bacteria to shift from using lactose , turning on the Lac operon. The inducer of the operon is IPTG. The experiment is successful if the bacteria grow, causing a change in the media color to blue as bacteria use lactose as an indication of operon gene function.

Day 18: The plates were checked for growth of colonies. The colonies were counted and recorded. This lab enabled students to visualize the genes turning of and on. The majority of the class time was spent dividing the students into four groups and beginning Lab #4 (Appendix A). Four different techniques to remove the pUC 18 plasmid from the transformed bacteria of Lab #3 were attempted The students used the remainder of Day 18 and all of Day 19 to try the plasmid removal process. It was expected that two to three samples of plasmid would be collected.

Day 19: The students continued to work on plasmid extraction. Group I

using the filtration method was able to collect only one sample. Group II used a boiling technique and collected two samples. Group II and IV used mini-prep and alkaline method and each collected three samples of plasmid. Each group had at least one microcentrifuge tube with a 40 microliter sample of possible plasmid in it.

Day 20: Lab #5(Appendix A) induced testing for plasmid isolation by using gel electrophoresis to isolate the pUC 18 plasmid and comparing it to known sample pUC 18 plasmid. Each group made 1.2% agarose gels and loaded separate wells with plasmid from Lab#4, known pUC 18 plasmid, running dye. The gel took about two hours to run.

Day 21: The gel of part 1 of Lab #5 was strained and refrigerated for 24 hours. The students began part 2 of Lab #5 which investigated restriction enzymes EcoRI and BamHI using lambda DNA. The students cut the lambda DNA first with EcoRI alone, then cut lambda with BamHi alone, and finally cut it with both enzymes. These three samples were loaded into separate wells of Pre-digested DNA(standard) and running dye were also loaded. The gels took about two hours to run.

Day 22: The bands in the gels of part 1 of Lab 5 were measured using a millimeter ruler and visually inspected to identify the presence of Puc18 in the sample from Lab #4. The gels from part 2 (day 21)

were dyed and refrigerated

Day 23: The gels of part 2 of Lab #5 were measured and all the data was recorded on semi-log graphs. Using the semi-log paper, mark the x-axis in 1 cm. intervals up to 5 cm. This axis represents the migration distance. The fragment size(in base pairs) is graphed along the y-axis.

Day 24: We discussed Labs #3, #4, #5. Stressing the continuity among the labs. The procedures and mechanisms for adding foreign DNA to the cell, turning on/off genes, and then removing the foreign DNA and finding it again were reviewed. We reviewed student results. This day gave the students the "Big Picture" of recombinant DNA research.

Day 25: We discussed the application of techniques like we had done in the lab. A discussion of insulin and interferon research was held. Also the future of this type research and possibilities for improvements in both plant and animal study were discussed.

Day 26: Reviewed the material in Chapter 11 for a test.

Day 27: Chapter 11 Test was given. The test contained 45 objective questions and two 10 point essays.(Appendix G)

Day 28: The students were given the day to work on the lab reports they had to write up individually on each of the five labs.

Day 29: The post test was given(Appendix D) in addition to the same 45 objective questions from the pre test, an additional essay was given

to evaluate the effectiveness of the labs. This essay question was not used for comparison with the pertest.

Audio-visual Aids used in Unit:

I found the more audio-visuals used in class to try to explain the concepts of the unit the better the material was received. The last three years I have been reviewing possible textbooks for the course and have seen a tremendous variety of supplementary material that would be used in this unit. I used a variety of overheads mainly to demonstrate difficult concepts.

Since the majority of the material taught in the unit cannot be seen, the use of pictures, charts, and diagrams is very helpful to the students to understand the structure and function of DNA. I used the chalkboard to draw the various structures of DNA, along with bonding assignments. I used overlays to describe the DNA replication process. Appendix B which contains other overlays began with the one of DNA replication(B-1 and B-2).

I found one of the most challenging processes to present to the students was that of protein synthesis. I have taught this process to first and second year biology students and have used a variety of special audio visual materials to help present this material including a felt board which contains colorful pieces of felt to represent the various components of the process. I also used overlays from a variety of sources. Appendix B contains overlays B-3 on the Central Dogma of Molecular Biology to give an overview of protein synthesis. Overlays B-4, B-5, and B-6 explain a specific aspect of the process of protein synthesis.

The third chapter, Chapter 11, on molecular genetics application required a number of overlays, B-7, B-8, B-9, and B-10 to help the student understand the operon control theory of protein synthesis. The final handout, B-11, was given to the students to fill in so they could better understand the function of the operon.

Pedagogical Value of Laboratory Exercises:

The purpose of the labs was to show that DNA can be isolated and manipulated. The basic nature of DNA has been introduced to and reinforced in students since elementary school but retains a mysterious quality. I showed in the lab exercises that DNA can be obtained as a visual mass and experiments can be done to show its properties.

In the first lab, DNA Isolation(Appendix A), the students were able to obtain DNA from tissue and accumulate a mass that could be seen. The lab gave the students the opportunity to homogenize thymus, filter it through cheesecloth, centrifuge, cool the precipitate and then spool the DNA strands on a glass stirring rod.

This lab permitted the students to see a mass of DNA fibers together. The lab brought the abstract concept of DNA to light. The students could know see and touch actual DNA stands.

Lab #2, Properties of DNA(Appendix A), continued the study of DNA by examining some of its properties. The students took the DNA from the glass rod and experimented with compounds and factors that might effect its structure. The students used a 1% NaCl solution, cold, and the enzyme DNAase to test effects on the DNA structure. The students used a 1% NaCl solution and tested the effect NaCl on the DNA spool ability after twenty four hours of exposure. The effects of heating and cooling DNA slowly as opposed to quickly were tested. The concept of "denaturation" was

clearly demonstrated. The contrast effect of speed cooling on renaturing of DNA was also shown. The final portion of the lab demonstrated the effects of restriction enzymes on DNA structure.

The completion and understanding of Labs 1 & 2 provided the background for the final three labs of the unit.

Lab #3, Bacterial Transformation of E.coli with pUC 18 plasmid(Appendix A) was the first of a series of three labs. I stressed to the students that in these labs they would be introducing a plasmid into a culture of E.coli then testing to see if the genes in the plasmid had transformed the bacteria to allow it to grow where it could not before. Working with the same transformed colony of bacteria, they would then turn off one gene and turn on another gene in the lac operon. Using the transformed culture, the students would then remove the plasmid in one of four possible ways and identify the isolated plasmid by using gel electrophorphoresis. I stressed to the students that success in Lab #3 would better the chances of success in Lab#4. Success with Lab #4 would greatly increase success in Lab#5. I reminded the students that this sequenced process is often used in research.

Lab #3, Bacterial Transformation, was a difficult lab for the students because they would not be able to tell if it worked correctly until the next day. The students used calcium chloride to make a colony of E.coli competent to take up the plasmid pUC 18. A discussion of the effects of ampicillin on bacterial growth was held prior to the introduction of two different colonies of E.coli on nutrient agar plates containing ampicillin. One of the colonies was labeled "minus DNA" and did not contain the pUC 18 plasmid and the other colony was labeled "plus DNA" carried the pUC 18 plasmid,

which contained the gene for ampicillin resistance. The plates were streaked in a similar pattern and allowed to grow for twenty four hours. The following day students could see the effect of incorporating pUC 18 plasmid on the bacterial growth on ampicillin. Many colonies had grown on the "plus"plate and very few on the "minus" plate.

After demonstrating that the E.coli containing the plasmid showed positive growth on ampicillin plates, the student next attempted to turn off one gene of the lac operon of the incorporated pUC 18 plasmid and at the same time turning another gene. This portion of the lab was difficult to prepare. X-gal(a histochemical substance which reacts with B-galactosidase to form a blue color) and the IPTG(an inducer of B-galactosidase activity in E.coli) were expensive and hard to work with. Therefore only a limited number of plates of nutrient agar/X-gar/IPTG/AMP could be prepared. Each group was given two plates, each inoculated with the transformed E. coli and incubated for 24 hours. After incubation a number of blue colonies appeared illustrating the bacteria's ability to use a new food source. The students calculated the efficiency of the transformation process from the data collected using the example explained in the lab procedure and from the class discussion held about the process.

The real success of the lab was that there was substantial bacterial growth on the plates. It was very striking to see blue colonies of transformed E.coli or a clear plate where the colony that you started with was not transformed. The concept of transformation and the lac operon were much easier to understand and visualize by the students with this lab.

The lab, Extraction and Purification of Plasmid(Appendix A), was the lab that gave the student the best opportunity to experiment on their own. My own research

techniques of plasmid removal from bacteria worked. I selected four different approaches for the students to try to remove the plasmid they had introduced in Lab #3 from the bacteria. I wanted to determine which was the most successful and which technique the students liked the best. All of the approaches were fairly successful. I divided the class into four groups and gave each group a different protocol to use to extract the plasmid. The students had a chance to try slightly different methods and to independent and investigative.

Approach I required a series of filtration through a .45 micron filter. This technique was a time consuming process that required close attention to detail. This approach took the students about two 50 minute class periods to complete. The students using this technique started two samples of transformed bacteria simultaneously so that they would have two samples to use in Lab #5.

Approach II was to lysis the bacterial cells by boiling. The technique was not as time consuming as the first approach but required the use of vacuum tubes to remove the liquid. The technique had to practiced many times by the group before they began their actual isolation. This approach took one 50 minute class period and the students were able to collect three samples with this technique.

Approach II was the lysis of plasmid removal which required a series of centrifugation. The technique was the shortest of the four, taking only about thirty minutes. The students were able to collect four to five samples of plasmid with this technique. The students seemed to like this technique the best because it had the fewest steps, took the least time, and resulted in the most samples of possible plasmid to be tested in Lab #5.

Approach IV used an alkaline solution to lyse the bacterial cell. The technique took about one hour to complete and the students were able to collect two samples during the time of this lab.

At the conclusion of this lab, all students had any where from two to five micro centrifuge tubes containing about 50 microliter of possible plasmid. Each group hoped that in the solution would be a quantity of pUC 18 plasmid.

The microcentrifuge tubes containing a clear liquid thought to contain plasmid were the only product produced in lab #4. Many of the students were a little unsure about having no other results. Labs done before in this and other science classes always seemed to produce a product that was visible or showed some change. The clear liquid in the tubes was not the type of results with which the students seemed to feel comfortable.

The final lab, Gel Electrophoresis of Isolated Plasmid DNA, had two parts. First, the students took plasmid isolated from Lab #4 and separated DNA by agarose gel electrophoresis. Students made their own 1.2% agarose gels and loaded the wells using 20 microliter micropipettes. The electrophoresis equipment and the power supplies used in class were home made. This portion of the lab was to show if the students had been able to isolate the plasmid from the transformed bacterial colony in the previous lab.

Secondly, the students worked testing the action of two restriction enzymes: EcoRI and BamHI on known phage DNA. This portion of the lab also used gel electrophoresis to separate the DNA fragments. A known DNA phage was digested with restriction enzymes and then loaded into wells of the gel. The DNA fragments moved through the gel via electrophoresis.

The lab required the introduction of new equipment to the students. I spent most of the first hour of the lab showing students how to use the gel electrophoresis tray, the power supply, loading the wells of the gel with the micropipettes, making the agarose gels and staining the gels.

On the second day of the lab, the students mixed glycerol with the samples of known and unknown(isolated from Lab#4) pUC 18 and running dye and loaded them into separate wells along with the running dye. It took about two hours for the running dye to separate out into three bands with the 24 volt power supply. The running dye is used to calibrate the agarose gel. The dye contained three different dyes, xylene cyanol: blue-green color equivalent to 2800 base pairs, bromophenol blue: purple-blue color equivalent to 250 base pairs and Orange-G dye: orange color equivalent to 70 base pairs.

The gel was run until the Orange-G dye was about 1cm from the end of the gel. The movement of each of the three dyes was measured and recorded for comparison with the bands of DNA fragments that would be strained.

The students returned after school to shut off the power and mark the distances the running dye traveled and begin the straining process. All the distances were recorded in millimeter lengths and placed on semi-log graph paper as previously described.

The students came in during lunch on the third day and loaded the wells with the various combinations of restriction enzymes and phage DNA. The students had the opportunity to try various combinations of restriction enzymes together with the phage DNA to try and get different sizes of DNA fragments. The restriction enzymes used in this portion of the lab were EcoRI and BamHI. One of the wells in the gel was loaded with predigested phage DNA. This phage DNA had been digested by HindIII and had

fragment sizes: 23.13, 9.14, 6.68, 4.36, 2.32 and 2.03. The bands measured in this well would become the standard to estimate the size of the DNA fragments in the other wells that had been digested by the other two restriction enzymes.

The regular class period was spent finishing the straining of the pUC gels which were then refrigerated for 24 hours. The students stopped the gels they had started two hours earlier an measured the distance of the running dyes.

On the fourth day of the lab, the students stained the gels with the restriction enzymes and studied the bands of the pUC 18 gel. They were able to calculate the size of the bands of the pUC 18 plasmid from Lab #4 from the data gathered. The students were able to see the band of known pUC 18 plasmid and then compare it to band, if any, of the unknown pUC 18 plasmid.

The fifth and final day of the lab was spent analyzing the second gel that contained the DNA fragments digested by the restriction enzymes. The students measured the bands that were strained and calculated the size of the DNA fragments. The procedure for the calculation of the fragments. The procedure for the calculation of the fragment sizes was the same procedure that was used for the previous lab part. Using the semi-log paper, the students established a slope line with the known DNA fragment that was digested by HindIII. The DNA fragments from the action of EcoRI and BamHI were then compared to this line and the estimated size of each fragment was recorded by the students.

This final lab exposed the students to many new terms and concepts, which were discussed in Chapter 11 of the textbook. A discussion of the mechanism of the restriction enzymes and how they work was the center focus of the lab. This lab allowed the

students to collect a wealth of data, graph it and calculate the size of DNA fragments. The work with gel electrophoresis showed how scientists can isolate process of DNA for study. The use of the equipment was the most interesting technique learned by the students. Their overall excitement and questions seemed to peak during this lab.

A student lab report was completed by each student individually at the completion of each lab. Each report included the student's personal reactions to the labs. As lab reports were written soon after the completion of the labs, I judged their comments to be fairly accurate about their true impressions of each lab. On the unit post-test, I asked the students to comment on the positive and negative aspects of the lecture and labs. I judged from these comments as well as the lab reports that the labs were well received and the basic concepts of each of the labs were understood.

I did not have any expectations of what the students would understand about any of these labs. From the interviews, I learned that the students knew very little about DNA structure, properties, and the behavior of restriction enzymes. The conclusions by the students written at the end of each of the labs made it clear to me that the basic concepts of the lab were understood.

Overall, I was very pleased with the labs, although they were too long. The unit needs to be shorter and since the lecture material is not going to shorten, the lab time needs to shorten. I need to rewrite a few portions of the labs to consolidate and save needed class time. The first two labs seem like the most likely location to consolidate initially. These two labs could be written in one multi-part lab.

Innovative Methods/ Demonstrations:

The only innovative demonstration used during the unit was the felt board(Appendix E) to explain protein synthesis, as discussed earlier. I developed it a few years ago to help students understand this process. The use of the felt board helps students remember mechanics of the process and build for the new principles of recombinant DNA. The felt board is a simple tool however the students seem to pay close attention to the colorful board and readily key in on the terms of protein synthesis. The students get an opportunity to use the board to explain to fellow students and further understand the mechanism of protein synthesis.

STUDENT TRANSFORMATION DATA

Pre and Post Test Results:

This was an Advance placement class that consisted of two males and nine females. The course is a shared time class with students of the following schools from Shiawassee County: Durand, Morrice, Perry, and Corunna. The students in the class had taken and Introductory Biology course and a second year Life Science course at their home schools which included Physiology/Anatomy, Genetics, Microbiology, Ecology or varied topics in Biology. The students were taking or had completed Chemistry.

These four area schools are considered rural in nature, and most of the students were in the top on-third of their respective classes. Each of the students came into the Advance Placement program with a slightly different background in science.

The pretest(Appendix D) that was given each student consisted of twenty-one multiple choice questions, nine true or false questions, and fifteen matching questions over the three chapters of the unit. The questions that were given included two basic types, simple recall questions and conceptual questions to test for the student's application of knowledge. Sixteen questions came form Chapter 9, fourteen questions from Chapter 10, and fifteen questions from Chapter 11. The relatively equal distribution of the questions was designed to test equally over all three chapters. The True-or-False type of question I realize gives the student a 50/50 chance at being correct but I believe that

overall it tells if the student has grasped the basic facts of the unit. The fifteen matching questions were divided into three groups of five questions with five answers for each question. This type of question allowed me to list a series of terms or people and give a series of statements of discoveries and see if the students can make the correct associations. The researchers studying DNA are important: this type of question allowed me to see if the students were cognizant of DNA research.

The twenty-one multiple choice questions were of two types: ten simple recall questions and eleven conceptual questions. The equal split of the questions would show whether or not the student could apply the subject matter.

The students took the pre-test the first day of the unit without being told in advance about it. I wanted a clear indication of their prior knowledge about the material in the unit without advance study. The test took the students about thirty-five minutes to complete.

The results of the test showed quite a gap among the students in their prior knowledge. The highest score on the pre-test was 29 and the lowest was 12 out of 45 questions. the mean score was 19.25 or 42.8% correct. The standard deviation was 5.74 and with standard error a value of 1.73.

The post test of the unit was the same test as the pre test. The questions were identical with the addition of an essay question to help me analyze if the students understood the material. The resulted of the post test showed improvement compared to the results of the previous test. The highest score on this test was 35 or 78%, which was obtained by the person who had received a 24 on the pre-test. The lowest score was 23 or 51% of the questions correct and this was not the student with the lowest score on the

pre-test. The mean score on the post test was 29.9 or 66.4.5 correct. The standard deviation was 3.15 with a standard error of .9497.

Teaching the unit showed a ten question improvement in the average score of the student. The students did not improve as much as I would have liked but there were positive signs that learning did take place.

A "T-test" was done to determine of the pre and post test were valid means of assessing the students progress. the "T-test" allows one to statistically accept or reject the Null Hypothesis. The Null Hypothesis in this case states that there is no difference between the unit's pre and post tests. The "T-test" uses the standard difference of the means and was determined by using the standard error of both samples.

The standard error of the pre-test was 1.73 and the standard error for the post test was .949. The calculation of the standard difference, the value was 1.96. The formula for the "T-test" is:

$$\frac{\text{ave of post test - ave. of pre test}}{\text{standard difference of error}}$$

$$\frac{29.91\text{-}19.25}{1.96} = \frac{10.66}{1.96} = 5.43$$

"T-test" value of 5.43

I calculated the degree of freedom to be: # taking 1st test + # taking the 2nd test minus two to locate the degree of freedom. The actual calculation was: 11+ 11 - 2 = 20. Using the distribution of the probability table, I found the value of twenty and went across to find the value of 5.42. The highest value of the table was 3.8, which was in the .001 column. The chances of these two sets of results being different because of probability are less than .1% of the time. I can reject the Null Hypotheses and say that

the data I gathered on the tests is valid and shows that the students did learn new material and show improvement.

I was quite pleased with these results. The mean average score of the students improved and I believe that the students did learn new material and apply it. All of the students did improve despite their disparate background.

I do not have any comparisons with students not taught the new curriculum. In subsequent years I will continue the pre-test practice and I do not plan to return to the old was of teaching. The group of students in the present class (1991-1992) showed a more significant increase in scores between the two tests.

Clinical Interview:

I conducted interviews with all eleven students following the pre test. I asked each student the same ten questions. The questions were designed to be very general and to give the students the opportunity to tell me if they knew any thing about the concepts and principles of the unit. A few of the questions were to determine if the students knew anything about the terminology of molecular biology. I interviewed each of the students privately so they would not feel any pressure from their classmates and I told them there were no grades being given for their answers.

The eleven students were given a private interview with the ten questions that took most students about ten minutes. I asked the students to please be candid with me and answer the questions honestly. The students had been in class approximately three months when the interviews were conducted. I sensed that most of the students were at ease with me and answered the questions honestly.

Five of the students were seniors and six were juniors. The grade point average of the class members ranged from 2.6 to 3.8 with and average of 3.25. Seven of the students interviewed indicated that they would like to study in science after high school.

A summary of their responses to the questions follows.

1.) What do you understand about genetics?

Five of the students said that they got genes from their parents for traits and

would pass these on to their children. The remaining students all had some response which included comments on the terms dominant, recessive, RNA, DNA, X and Y chromosomes, and genes. One student expresses high level of confidence with her understanding of genetics and her scores were the highest on the pre and post test.

2.) How do you define DNA?

Eleven different responses to this question were recorded. Three of the answers contained some misconceptions about DNA. One student said it "contains chromosomes", another student said it "contains genes" and a third said it contains "proteins with and case ending".

3.) What can you tell me about the following terms: genes/nucleotide/chromosomes

I expected that chromosomes would be the best understood, followed by the concept of the gene and the least understood would be the nucleotide. Eight of the eleven students gave and appropriate definition of the chromosome. Ten of the eleven seemed to understand the concept of a gene. Only two students gave a response to the term "nucleotide".

4.) What do you understand about protein synthesis?

I had expected that the majority of the students would understand the basic framework of the protein synthesis process involving DNA, mRNA, tRNA, and ribosomes. Only a surprising two students of eleven students could define and summarize the process. Four students could define protein synthesis and five students seemed to have no ides what it was. I was quite disappointed with these responses because I had taught some of these students for two years and had spent time each year on this topic. The results of this question indicated that I would need to spend more time on Chapter

10 and protein synthesis in the unit.

5.) Can you name anyone who has done research with DNA?

I was disappointed in their lack of knowledge of names associated with this topic. The name of Mendel was mentioned by three students. Watson/Crick was mentioned by two other students and two other students said the name of Cook, a name that I had not heard associated with Genetics. Four of the students could not think of anyone that did research in the field of genetics.

6.) What do you understand about the science of recombinant DNA? What does "recombinant" mean?

This subject had not been taught before and I wanted to see if the students had picked it up from the media. Seven of the students had a good idea of what recombinant DNA was all about. Four of the students did not even try to explain it. These responses were encouraging because at least some of the students had some basics upon which to build.

7.) What is a virus?

Only two of the eleven students could answer this question with specific and correct responses. This question showed that many of the students have misconceptions about the viral DNA and RNA and replication. The answers included responses like: "it's a cell", "it's a thing that causes diseases" and "it floats in the air and can make you sick."

8.) Do you think cancer & genetics and connected at all? If so how?

I was pleases to see that if the student thought about it, they could make a connection between genetics and cancer and explain it using the appropriate terminology.

Four of the students were not sure what the specific connection between cancer and genetics was, but knew there was a connection. No one though was not able to see the connection.

9.) If you had to define "biotechnology", what would you say?

This question was to give me an indication of the parts of Chapter 11 on Biotechnology they already understood. Three of the students could define the term properly and their answers included: "to create new living things", "modify living cells", and "understand detail aspects of biological systems". Eight of the students could not define the term or had the wrong concept.

10.) Can you tell me what you think is important about genetics?

All of the students had an answer to this question. Four of the eleven saw the importance of Genetics to be in the understanding of diseases and how to correct them. Other answers included" to discover how to clone organisms", "to modify our life", "to study behavior of people" and finally "its the new frontier of science."

The pre test interviews indicated that the level of understanding of the field of molecular biology was limited. I discovered that the students had a basic notion of what DNA was but did not understand the structure of it. The subjects of recombinant DNA and biotechnology were new and unfamiliar to most of the students. The students knew little of the research of researchers in the field of molecular biology but all saw the field to be important in the study of science.

These findings indicated that I needed a thorough review of basic biology including DNA, nucleic acids, protein synthesis and possibly reinforcing lab work. I decided that I needed to progress slowly through Chapters 9 and 10 to build the student's

knowledge. The labs accompanying Chapter 9 and 10 reinforced this information. The isolation and properties of DNA lab helped the students visualize the concepts of the chapter. The major portion of the unit was contained in Chapter 11, Biotechnology. The three labs on bacterial transformation, plasmid removal, and gel electrophoresis tied together the concepts of biotechnology.

The pretest interviews indicated that the unit would take more time than the original five weeks that I had planned. Therefore I allotted more time to reinforce Chapter 9 and 10 than I originally planned.

Subjective Evidence of Effectiveness of the Unit:

The students gave me their thoughts on the significance of the five labs of the unit on the final essay question of the post test. I did not grade this essay and I told them this in hopes that they would feel free to respond candidly.

The question was written "a post interview" to see how much of the unit information was acquired from the labs. The question was also intended to see if they understood the significance of the labs and equipment used. I wanted to give the students the opportunity to write down all of their thoughts about the unit without feeling pressured by me in a clinical interview format.

The final question was:

"Explain the significance of the 5 labs done during this unit"

Students were given the following specific questions:

A.) How did they fit into the chapter material?

Eight of the eleven students could cite a specific example from the labs that related to the chapter material. Five of the students described how that illustrated the concept of transformation. Four students discussed the relationship between lecture material on restriction enzymes and its use in the lab. Overall the comments were very reflective of the cooperation between lab and lecture. Two students stated the following comments on the coordination between lab and lecture:

"showed how to study molecular biology","Lab explained the concepts discussed

in the chapter","see how scientists went about finding information" and "hands on experience with concepts from the book".

B.) What did you attempt to do in them?

Six of the eleven students listed cutting the DNA and inserting pUC 18 into the bacterium. Five of the students mentioned gel electrophoresis of DNA fragments. Other comments from students were on the procedures done with the bacterial transformation experiment including competent cells, inducers, inhibitors, restriction enzymes and operon genes. One student wrote the following about the concepts learned

"We didn't just read about someone's experiment, we did them. It was exciting to see growth on the plate, working with something scientists just recently started playing with."

C.) Did you see any relationship between the labs? If so how?

I wanted to see if the students could see the sequence of labs and understanding how they related to each other and the unit. All the students made positive concepts about the relationship between the labs. Some of the comments included:

"All labs dealt with plasmids and cutting DNA", "Just like big long experiment", "Each lab laid down the groundwork for the next one" and "Products from one lab were the materials for the next"

D.) What scientific principles did you learn from them?

All of the students listed at least two scientific principles. The three principles mentioned by a majority of the students were restriction enzymes; DNA restriction, insertion, and isolation; and electrophoresis of the gels. Other students commented on recombinant DNA, bacterial transformation and plasmids.

E.) Were there any special skills you acquired as results of doing the labs?

Five students cited electrophoresis work including loaded wells and measuring and staining gels. Two students mentioned making electrophoresis gels, using micropipettes and microcentrifuge tubes, and inserting plasmids into bacterial. One student summed it up this way: "I learned that it works and will not always give the same result to each group, also it takes patience to get it to work."

It was very rewarding the read comments like the one above. I think that besides learning about scientific technology and DNA terminology, the students also learned much about the scientific method and scientific patience. The labs seemed to provide students with a realistic look at performing an experiment in a real life situation.

DISCUSSION AND CONCLUSION

Aspects of the Unit that are Effective:

The aspect of the unit that was most effective was the labs. The students seemed genuinely interested in the new technology that I was presenting. They were unusually quiet during the explanations for the labs. The newness of the labs and the equipment was the primary reasons for their interest. The number of the students in each group who got actively involved in each lab was greatly increased compared to other classes. In my other lab classes, a usual lab group of four students working together involved one or two of the students doing the work and two recording the data or just watching. The labs in the molecular biology unit seemed to involve all of the students in the experiments. During the final three labs there was not a student who did not load a well or stain a gel or measure a band of DNA on a gel. Students who were generally reserved in most labs were actively involved in performing these labs.

Discussions during the labs centered on the experiments and the results they were getting, not normal classroom talk. Often during other labs I have to remind students to stay focused on the task at hand and to discuss what they were doing with each other. I was pleasantly surprised that I did not have to do this with these labs. I actually had students come up to me to ask questions about the labs and their future applications in society. These kind of positive experiences, I would like to have with all labs. The

students actually came into class early and immediately went to work of would get their results from the previous day's lab. Many students would come in before school, others would stay after school, and some came in during their lunch period or study hall to work on the labs. The excitement and concern by the students gave me the feeling that this was the most effective part of the unit.

I would like to make the final three labs more open ended than they are. There are opportunities to open the labs for independent research. The students seemed interested in the labs and with some options at the end of the lab the students could go further and discover more. For example, Lab #4(Appendix A) gives the students four options of plasmid removal. In the actual lab only one option is tried by each of the students. I would like each student to have the opportunity to try the other three plasmid removal techniques. The students then could revise them of possibly combine them to a more effective plasmid removal technique. Lab#5(Appendix A) could be expanded by increasing the types of restriction enzymes used in the experiment. The lab could also be used to show the separation of proteins as well as DNA.

The equipment used in the labs needs to be more extensive and precise. Most of the equipment used in the labs homemade in our lab. I would like to purchase more commercially made equipment.

The audio visual materials used were adequate to explain the material. I would like to find a good video tape that demonstrates some of the techniques needed for the unit. I think that often times students listen better to others than to their teacher when new materials is explained.

The aspects of the unit that need improvement include the chapter on protein

synthesis. I have an adequate amount of material to explain the process but I would like to find a good video tape that shows the sequence of events that occur simultaneously but a video could do a better job of this.

I think that it would be possible to combine Lab #1 and Lab #2(Appendix A) together. These two labs are similar and by combining the students would accomplish the same objectives and save some additional time in the unit for other procedures.

I need to acquire more lab equipment so the lab groups could be smaller. Ideally, a group of two students would work the best. The equipment is small and its difficult for all of the students to see the procedure when the group is as large as four or five students.

The use of weekly quizzes over the lecture and lab material would help the students keep up with the material weekly instead of putting off review until the unit test. The use of weekly quizzes might improve the test results on the chapter tests.

Overall Evaluation:

My evaluation of the unit was that it was very successful. Each day of the unit, I left the class excited to come back the next day to continue the work. My excitement was mirrored by my students trying to learn new techniques, new terminology, and apply them in lab. I think the students learned a great deal about molecular biology. Learning the material was important but also they gained an appreciation for the future of molecular genetics. I think they are literate enough to pick up a newspaper, magazine, or watch a newscast and understand the meaning of new discoveries in molecular biology. The geome human unfolding project can be understood by my students.

I think the students got genuinely excited about science and especially molecular genetics. Excitement in the classroom is something I do not often see in teaching. Their excitement with science of molecular biology was the biggest accomplishment of the unit. This excitement carried on through to the following other units.

APPENDIX A

LABORATORY EXERCISES

EXPERIMENT #1: DNA ISOLATION

Background Information:

To understand the eukaryotic cell at the molecular level requires isolating the nucleus. We must rupture the cell membranes in a controlled fashion. If careful, you can remove the soluble components of the cell and separate them from the nucleus. The nuclei can be separated by low speed centrifugation from the rest of the cell components.

Objective:

To isolate DNA from the nuclei of eukaryotic cells

Materials:

Calf Thymus - 15 grams
Cold Denatured Alcohol
Glass vials/Test tubes
Glass Rods
Micropipets(100 ul)
Sodium Dodecyl Sulfate (SDS)
Nuclear Stain
Cheese Cloth
Nuclear Buffer
Ice Bath
Microscope
Microscope Slides
Clinical Centrifuge
Blender
Funnel
500 ml Beakers
Toothpicks
Scisssors

Procedure:
PART I - Isolation of Nuclei from Cells

1. Place the 15 grams of calf thymus into a 500 ml beaker-cut into less than 1 cm pieces with scissors

2. Place one section for every pair of students into a beaker,seal it, and refrigerate for part III and the remaining sections in the blender.

3. Add 100 ml of cold nuclear buffer into a blender

4. Add 12 Ice chips, blend for one minute at high speed

5. Filter the homogenate through two layers of cheese cloth

6. Pour the homogenate into test tubes and centrifuge for 5 minutes at 2000x rpm

7. Carefully pour off and discard the supernatant(top)- the pellet at the bottom contains the nuclei

8. Transfer the nuclear pellet to the blender with 100 ml of cold nuclear buffer

9. Blend the pellet for 10 seconds - refilter with cheese cloth

10. Store the mixture in the refrigerator until needed

PART II - <u>DNA ISOLATION</u>

1. Place 2 ml of the nuclear suspension in a test tube

2. Add 1 ml of 1% SDS solution into the vial/test tube note the appearance and consistancy

3. Allow to sit for 5 minutes

4. Pour 3 ml of cold denatured alcohol(on top)

5. Dip the glass stirring rod through the solution and slowly rotate - JUST DNA will spool on rod

Part III - Microscopic Analysis

1. Take two clean microscope slides

2. Take a clean toothpick,scrape the surface of the saved thymus section and smear on both microscope slides. Allow to air dry

3. Using a micropipete add one drop of nuclear suspension(part I, step 10) to the center of the slides, smear-air dry

4. Place three drops of alcohol on both slides to fix the tissue to the slide. After 2 minutes rinse with water with slide inverted.

5. Add three drops of nuclear stain, allow to stand for 5-10 minutes - rinse by inverse wash of water

6. Air dry for a least 10 minutes and examine under high power(43x). Nuclei should be purple,cytoplasm pale blue, nucleoli will appear small dark dots in the nuclei.

Results/Data Collection

Draw a picture of the nucleus view under the microscope at high power.

Interpretations/Conclusions

1. Make a list of five comparisions in the morphology of the isolated nuclei to the intact thymus cells

2. SDS solution is a detergent that dissociates histone proteins from DNA. Describe the change in the clarity and viscosity of the nuclear suspension when SDS was added.

EXPERIMENT 1 - DNA ISOLATION

Teacher's Guide

Time Frame: One Class Period(50 minutes)

Target Group: 2nd-3rd Biology Students

Prepartation of Materials:

1. Calf Thymus Sample: 15 grams of fresh thymus
   Which can be obtained from a local butcher shop or meat dept

2. Nuclear Buffer(Modern Biology Kit #1)
   Magnesium Chloride 1M
   Sodium Chloride (1% solution)
   Nonidet P-40 1M
   Tris pH 7.5 : .19 grams of Tris HCl
   .09 grams of Tris Base
   .07 grams of EDTA
   mix in 200 ml of distilled water
   Dilute 5ml of concentrated buffer in 500 ml of distilled water - store in refrigerator
   The above chemicals come as a kit,formulas by writing Modern Biology Inc.

3. Nuclear Stain: 1% Methylene Blue(1 grams/100 ml)

4. Sodium Dodecyl Sulfate(SDS):1 grams/100 ml of water

Sources of Materials:

Modern Biology Inc.
P.O. Box 97
Dayton, Indiana 47941-0097
1-800-7336544

Biochemical Stores
Biochemistry Bldg.
M.S.U.
East Lansing,MI.
48824

Sigma Chemical Co.
P.O. Box 14508
St. Louis,MO. 63178-9916

Comments: This lab is a good visual lab for the students to see DNA in the cell and compare what they see on a glass rod to under the microscope, which is nuclei of the cell.

References:

Anderson, John; Modern Biology Series, Purdue University,1987.

Sambrook, Fritsal, Maniatis; Molecular Cloning; 1989.

Advance Placement; Laboratory Manual of Students; The College Board, 1989.

EXPERIMENT 2 - PROPERTIES OF DNA

Background Information

A single human chromosome DNA molecule is about 40 cm long. Isolating DNA can be done by adding alcohol to a homogenized cell in a test tube. The DNA fibers can be precipitated and spooled on a glass rod.

DNA is an extremely long molecule that is very thin,yet quite rigid. The isolation of DNA is difficult because the long rod like molecules can be broken. DNA can be broken down by an enzyme called deoxyribonuclease I or DNAseI. THis enzyme breaks the bonds between the nucleotide units in the DNA.

Objective: To study the structure of DNA and the factors that denature it, particularly DNase

Materials:

Cold Denatured Alcohol
Bottle of blended Calf Thymus
DNAse I(Modern Biology Kit #1)
Glass Vials/Test tubes(1 per 2 students)
Glass Rods (1 per 2 students)
transfer pipettes
2 Large transfer pipettes
ice bath
hot plate/bunsen burner
1% NaCl soltion

Procedure:

Part I - DNA Spooling

This portion of the lab is similar to Lab #1 part II

1. Place 3 ml of blended thymus into a glass vial/TT

2. Carefully pour 3 ml of cold alcohol into vial; the alcohol should form a layer on the top of the solution.

3. Dip the glass rod into the vial and slowly rotate the rod and raise it into the alcohol. Fine fibers should form on the end of the rod.

4. Record the observations of the DNA on the rod.

Part II - Reverse the DNA Precipitation
(Putting DNA back into solution)

1. Place the rod with DNA fibers into a test tube

2. Add 2ml of 1% NaCl, shake the rod until the DNA fibers are in solution

3. Let stand for 24 hours

4. After 24 hours attempt to respool the DNA on the glass rod by first adding 3 ml of cold alcohol.

5. Record your observations of the DNA on the glass rod

Part III - Analysis of Denatured DNA

1. Place a new sample of 3 ml of DNA solution in the glass vial/T.T.

2. Hold the vial with a test tube holder and boil carefully for 1 to 2 minutes

3. Place the heated solution in an ice bath to cool quickly for three minutes

4. Add 3 ml of cold alcohol to the solution

5. Attempt to spool the DNA with a glass rod.

6. Record your observations of the attempt to spool

7. Repeat steps #1 and #2 with a new DNA sample.

8. Cool the solution slowly at room temperature for five minutes

9. Add 3 ml of cold alcohol to the solution

10. Attempt to spool the DNA with a glass rod

11. Record your observations of the attempt to spool

Part IV - Effects of DNAse I on the DNA

1. Place a new sample 3 ml of DNA solution in a glass vial/ Test tube

2. Using a micropipette add 50 ul(about 4 drops) of DNAse I and mix

3. Let it stand for 10 minutes, then add 3 ml of cold alcohol

4. Attempt to spool the DNA on the glass rod

5. Record the results of the attempt to spool.

6. Stir the alcohol and DNA mixture together

7. Record the color and appearance of the mixture

Results/Data Sheet: Make a chart to coordinate the data from the many observations made. Below the results, list the factors that influence the results.

Interpretations:

1. List the basic properties of DNA as observed in lab

2. Describe and explain the action of the DNAse I on the DNA.

EXPERIMENT #2 - PROPERTIES OF DNA

Teacher's Guide

Time Frame: One Class Period (50 minutes)

Target Group: 2nd - 3rd year Biology Students

Preparations of Materials:

1. Calf Thymus DNA solution: 15 grams of fresh calf thymus blended at high speed with 100 ml of 1M magnesium chloride, 1% NaCl, Tris buffer (pH 7.5) in 100 ml of distilled water.
   (same as the DNA solution from Lab #1)

2. DNAse I : 500 ul I of DNAse I suspended in Tris buffer of pH 7.5

3. Tris Buffer: .19 grams of Tris HCl
   .09 grams of Tris Base
   .07 grams of EDTA (mix in 200 ml of distilled water)

4. 1% NaCl Solution: 1 gram of NaCl in 100 ml of distilled water

5. Denatured Alcohol: 200 ml of 90-100% ethyl alcohol

Sources of Material:

Modern Biology Inc.
P.O. Box 97
Dayton, IN 47941-0097

Sigma Chemical
St. Loius , MO.

Carolina Biological Supply
Burlington, North Carolina

Comments:

This four part lab can be done in one class period with a little advance prep time. From this lab, the student should get a good overall picture of the properties of DNA. Actually this lab could be done by first year students to help explain the basic properties of molecular genetics.

References:

Anderson, John; Modern Biology Series, Purdue Univ., 1987.

EXPERIMENT #3 - BACTERIAL TRANSFORMATION
OF E. COLI WITH pUC 18 PLASMID

Background Information:

The bacteria Escherichia coli (E. coli) is an ideal organism for molecular biologists to manipulate and is used extensively in recombinant DNA research. It easily can be grown in luria broth(L.B.) or luria agar(L.B. agar). The single circular chromosome of E. coli contains 5 million DNA base pairs, only 1/600th of the total amount of DNA in a human cell. In addition, small circular DNA molecule called PLASMIDS also carry genetic information. The plasmids are extrachromosomal; they exist separately from the chromosome. Under normal circumstances, plasmids contain genes that enable bacteria to survive and grow in certain environments. Some plasmids carry one or more genes that confer resistance to antiboitics to the bacterium.

Bacterial transformation is the uptake and expression of foreign plasmid DNA by the recipient bacterium that can result in conferring a particular trait to the recipient. The transformation occurs when the bacteria is in the growth stage called COMPETENCE,when they are most receptive to foreign DNA uptake. Competence to incorporate DNA develops in time. Competency is achieved by treatment with calcium cations.

Objective: To introduce the pUC18 plasmid into E.coli in order to create a population of bacterial cells resistant to ampicillin and action of the operon gene "lac operon" by bacterial transformation.

Materials:

plasmid pUC18
ampicillin
luria broth(LB broth)
luria agar(LB agar)
inoculating loops
micropipetes (10 ul, 100 ul)
sterile microcentrifuge tubes
calcium chloride solution (2% solution)
test tubes/test tube racks
E.coli(JM101 strain)
X-gal/IPTG/Amp LB plates
water bath (37 degrees)
Ice bath
Incubator (37 degrees)
glass pasteur pipette

Procedure:

Part I - Prepartation of Competent Cells

1. Place 5 ml of a 2% calcium chloride solution into a test tube and place in an ice bath.

2. Add 100 ul of E.coli to the calcium chloride/mix

3. Incubate the cells for 10 minutes on ice,cells should be competent at this stage

4. Cells can be stored in refrigerator for up to 24 hrs

Part II - Uptake of DNA by the Compotent Cells

1. Take two microcentrifuge test tubes,label one "+" and the other "-"DNA.

2. Place the tubes on ice for 5 minutes.

3. Transfer 10 ul of plasmid pUC 18 to the test tube labeled "+" DNA

4. Transfer 50 ul of the competent E.coli to both positive and negative microcentrifuge test tubes and swirl

5. Store both tubes on ice for 15 minutes

6. Transfer both tubes to 37 degree water bath for 5 minutes

7. Add .7 ml or 700 ul of LB broth to both microcentrifuge tubes. Incubate for 30 minutes in the 37 degree water bath.This allows the bacteria to recover from the procedure and begin to express the plasmid's genes

8. Obtain two ampicillin LB plates(LB+). Label one "+" DNA and the other "-" DNA

9. Transfer 200 ul of bacterial suspension from each microcentrifuge tube to the appropriate plate: "+" to "+" and "-" to "-"

10. Using a modified pasteur pipette/ loop
    spread the bacteria evenly over the agar

11. Let the media stand at room temp. for 30 minutes

12. Invert the plates and store in incubator 37 degrees

NEXT DAY: count the colonies on each plate and record

Part III - lac Operon Expression

The pUC18 plasmid besides confering resistance to ampicillin can also transform E.coli to use a different energy source for growth. When the bacteria is grown on LB+ plates with X-gal/IPTG(a histochemical substrate which forms a blue-colored product and an inducer of the activity), the bacteria stops using galactose and uses lactose as the energy source. The inducer IPTG turns on a new gene on the plasmid to operate. As the bacteria grow and uses lactose they will turn the media blue, as an indicator of the bacteria transformation.

1. Using the same cells from part IIstep 4(Compenet +),
   place a 200 ul of the transformed cells on
   LB X-gal/IPTG/Amp plate.

2. Smear the culture evenly over the plate using a
   sterile modified pasteur pipette.

3. Allow the smear to sit for 30 minutes then incubate
   inverted for 24 hours.

NEXT DAY: Count the number of colonies that grew on the
experimental X-gal/IPTG/Amp plate and record
number and appearance of colonies

Results/Data Sheet:

1. Draw a picture of the three plates used in parts II
   and III,label each drawing and describe the events
   that occured.

2. Count the nuber of visible colonies on each plate:
   Minus DNA: _____
   Plus DNA: _____
   LB X-gal/IPTG/Amp: _____

3. Calculate the transformation efficieny of the LB/X-gal/IPTG/Amp plate. Using the following formula

Formula: # of colonies on plate X $\frac{\text{total vol. of mix}}{\text{total vol. of mix used on plate}}$

Example Problem:
If you observed 100 colonies on your plate, the efficiency is calculated as follows:

$$(100 \text{ colonies on plate}) \times \frac{300 + 600 + 10 = 910}{200 \text{ sample used}} = 455$$

Note: total mixture=300 ul competent cells+ 600 ul of LB broth + 10 ul of pUC18

100 colonies x 4.55 = 455 colonies expected from total reaction mixture

455 colonies/30 ng plasmid DNA = 15.1 colonies/ng plasmid DNA
(30 ng is concentration of plasmid DNA used)

15.1 colonies/ng x 1,000ng/mg=1.51x 104 colonies/ul g plasmid used

Therefore, the transformation efficiency in this example would be on the order of 104 per mg of plasmid used

Interpretations/Conclusions

1. Explain how and why the cells can be resistant to ampicillin and have the lac Operon too?

EXPERIMENT #3 BACTERIAL TRANSFORMATION OF E. COLI WITH pUC 18 PLASMID

TEACHER'S GUIDE:

Time Frame: two day lab: Parts I and II(first class)
Part III (second class)

Target Group: 2nd - 3rd year Biology Students

Preparations of Materials:

1. 2% Calcium chloride solution

2. E.coli suspension: strain JM101

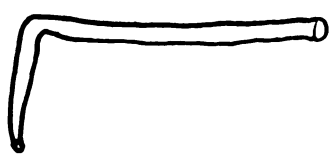
3. pUC 18 plasmid: 30 ng plasmid in suspension
(Modern Biology Kit #3)

4. LB broth: 10 grams of tryptone
10 grams of sodium chloride
5 grams of yeast extract
.1 gram of NaOH
1 liter of distilled water
Autoclave for 15 mins at 121 degrees and 15 psi

5. LB plates: use 500 ml of LB broth and add 7.5 grams of bacto-agar and combine if both sterile, need to heat to boil and pour in sterile petri dishes.

6. Ampicillin(25 mg of Amp/ml): add .5 grams of ampicillin to 25 ml of distilled water and pass through a sterile diposable .45 micron Millex filter collect in 1 ml samples which can be frozen until needed. (.22 micron Millex filter works better)

7. Pasteur pipette: heat bend the narrow part of the pipette as shown below. Caution:glass heats quickly



8. LB X-gal/IPTG/Amp plates: 500 ml of LB+ agar, autoclave,as cools add 2.5 ml of 2% X-gal and add .5 ml of fresh 100mM IPTG(Isopropyl b-d-Thiogalacto-pyranoside),1 ml of Ampicillian & pour into sterile petri dishes

9. 2% X-gal: .16 grams of X-gal(5-Bromo-4Chloro-3-Indolyl b-d-Galactopyranoside) in 8 ml of DMF solution (N,N Dimethylformamide)

10. 100mM IPTG solution: .07 grams of IPTG in 3 ml of sterile water

Sources of Materials

Modern Biology Inc.
P.O. Box 97
Dayton, IN. 47941-0097

Sigma Chemical Co.
P.O. Box 14508
St. Louis, MO. 63178-9916

Biochemical Store
Biochemistry Building
Michigan State University
East Lansing, MI. 48824

Carolina Biological
Burlington,N.C. 27215

Comments:

This lab requires a lot of explanation and discussion of DNA, plasmids, recombinant DNA, cloning, bacterial transformation, and nucleotide sequences AHEAD of time.

The lab will work well if done two consecutive days. It can be broken up between parts II and III, because each will take about one class period to do. The lab can be shortened if the teacher makes the cells competent before class starts.

References:

Anderson, John; Modern Biology Series; Purdue Univ.; 1987.

Sambrook, Fritsel, Maniatis; Molecular Cloning; 1989.

Helms, Doris and Phillip, Corker; Preparator's Guide; Worth Publishers; 1989.

Advance Placement Biology; Laboratory Manual; The College Board; 1989.

EXPERIMENT #4

EXTRACTION & PURIFICATION OF PLASMID

Background:

The removal of a plasmid from a bacteral colony can be done by a variety of techniques, including boiling, pH changes(alkalanity) and filtration. The lab on plasmid extraction has four parts. You will be assigned one of the four techniques to remove the plasmid(pUC18) from the colony of E.coli you used in experiment #3.

Objective: To extract the pUC18 plasmid from E.coli cells that have shown they possess the plasmid

Materials:

overnight bacterial culture of E.coli with pUC18
four 15 ml screw top pyrex or disposal test tubes
two Millex 0.45 micron filters + holder
one 1/16 inch female luer adapter
two 20 ml syringes(with luer locking fitting)
two 30 ml luer locking fitting syringes
single layers of 4"x4" cheese cloth
pastuer pipette with bulb
ice bath
10% flocculating agent(polyethylenimine)
TENS solution
50 ml beaker
centrifuge
precipitating agent(potassium acetate)
100% isopropyl alcohol
TE buffer

Procedure I: Filtration Process

1. Remove a 15 ml sample of an overnight culture of E.coli to a sterile 15 ml tube

2. Cap the sample and invert five times - observe consistence of the solution

3. Add 4 drops of flocculating agent to sample/invert the tube five times - clumping of sample should begin.

4. Place the tube in an ice bath for 10 - 20 minutes to allow the clumps to settle - DO NOT DISTURB

5. After the clumps have settled out, use a pasteur pipette to remove the clear broth down to the 4 ml mark on the test tube. Discard the liquid.

6. Swirl the concentrated colonies to resuspend them

7. Add 3.5 ml of TENS solution to the supension, cap and gently invert the tube ten times.

8. Increase the volume from 7.5 ml to 12 ml by adding 4.5 ml of precipating agent,cap, invert 10 times stand at room temperature for five minutes

9. Place four layers of cheese cloth over the 50 ml beaker and push down in the center to form a funnel shaped area.

10. Carefully pour the solution through the cheese cloth and discard the material and cheese cloth but save the liquid.

11. Remove the plunger from the 20 ml syringe and attach the barrel of the syringe to a .45 micron filter. Place the other end over a new clean 15 ml test tube, pour the solution into the barrel of the syringe and insert the plunger and SLOWLY push all solution through the filter.

12. Add 6 ml of isopropyl alcohol to the test tube of solution, cap the sample and invert 10 times. Let the tube sit at room temperature for 5 minutes.

13. Connect a clean 20 ml syringe to a new clean .45 micron Millex filter. Remove the plunger. Place the other end over a clean 15 ml test tube to collect wastes. Pour the solution of #12 step into the syringe barrel, insert plunger and push SLOWLY the solution through. The plasmid should be collected on the filter, on the surface closest to the syringe.

14. After removing the filter from the syringe, draw 20 ml of air into the empty syringe and connect syringe to the filter. Push the air through the filter. Push the air through the filter to remove the remaining liquid.

15. Remove the 20 ml syringe from the filter

16. Remove the plunger from a 3 ml syringe and attach it to the .45 micron filter.

17. Attach a 1/16" female luer fitting coupling to the other end of the filter.

18. Connect another clean 3 ml syringe with plunger into this female luer coupling.

19. Add 1 ml of TE buffer to the open barrel syringe, insert plunger and push SLOWLY the buffer solution through the filter into the other syringe. It helps if you slowly PULL the opposing syringe's plunger at the same time as you are pushing, back and forth

20. Push the solution through the filter 10 times.

21. Remove the original(1st) syringe with the solution and inject it into a clean microcentrifuge tube to save for Experiment #5.

22. The sample should contain 5 to 30 micrograms of pUC18 plasmid in it, which will be saved for Lab #5

CLEANING: syringes, tubes, and equipment can be rinsed in water. .45 micron filter are reuseable and need to be cleaned with alcohol by using the syringe to rinse through.

Procedure II: Boiling Technique

Materials:

overnight culture of E.coli
LB broth
Microcentrifuge
Microcentrifuge tubes
STET solution
Lysoszyme solution
2.5M sodium acetate solution (pH 5.2)
isopropanol
70% ethanol
micropipettes
vacuum source(water aspirator is fine)
TE buffer
sterile toothpicks
vortex mixer (optional)

1. Remove a 1.5 ml sample of an overnight E.coli suspension and place in microcentrifuge tube.

2. Centrifuge the tube at high speed for 45 seconds and store the remainder of the culture in the refrigator.

3. After centrifuging, the bacteria will be a pellet on the bottom of the tube. Connect a length of rubber tubing to the vacuum source and insert a disposable pastuer pipette into the other opening of the tubing. Remove the liquid medium in the microcentrifuge tube by gentle aspiration. Touch the tip of the pipette to the surface of the liquid and keep the tip as far as possible from the pellet of bacteria. Vacuum the walls of the tube to remove any drops of liquid.

4. Add 350 ul of STET solution to the tube and resupend the bacteria.

5. Add 250 ul of freshly prepared lysozyme solution which has been held on ice. Resuspend by vortexing for 3 to 5 seconds or shake gently to resuspend.

6. Place the tube in a boiling water bath for exactly 40 seconds.

7. Centrifuge the solution for 10 minutes in the micro-centrifuge.

8. Using a sterile toothpick remove the pellet of cell debris from the bottom of the tube.

9. Add 40 ul of 2.5M sodium acetate and 420 ul of isopropanol. Mix by vortexing or shaking and store for 5 minutes.

10. Centrifuge the solution for 5 minutes

11. Remove the liquid by aspiration (step #3)

12. Invert the microcentrifuge tube on paper towel until all traces of fluid are gone.(Up to 10 mins)

13. Add 1 ml of 70% ethanol. Centrifuge for 2 minutes

14. Remove the ethanol by aspiration and store the tube until all liquid has evaporated.

15. Resuspend the plasmid in 50 ul of TE buffer. Typical yield of 4 mg per millilter of bacteria should be produced.

16. This plasmid will be assayed in Experiment #5

Procedure III: "mini-prep"

Materials:

Overnight culture of E.coli with plasmid
TENS solution
TE buffer
3M sodium acetate
100% ethanol
70% ethanol
micropipettes

1. Place 1.5 ml of the overnight E.coli culture in a micro-centrifuge tube and centrifuge for one min.

2. Discard all the supernatant fluid except the last three drops. Vortex or mix to resuspend the cells.

3. Add 300 ul of TENS solution to the suspension and mix or vortex. This solution is basic and will lysis the cell wall of the bacteria and denature the DNA.

4. Add 150 ul of sodium acetate to neutralize the solution and renature the DNA. Mix for 10-20 seconds

5. Centrifuge for two minutes to pellet the cell debris. The DNA will remain in solution.

6. Pour the supernatant fluid into a clean test tube and add 0.9 ml of 100% ethanol that was in the freezer overnight.

7. Centrifuge for two min., a white pellet will form.

8. Discard the supernatant fluid and rinse the pellet twice with 70% ethanol.

9. Air dry the pellet for 5 minutes.

10. Add 40 ul of TE buffer to resuspend the pellet.

11. Save the microcentrifuge tube with sample of the plasmind for experiment #5.

Procedure IV: Alkaline Method(regular prep)

Materials:

overnight culture of E.coli containing pUC18
centrifuge
microcentrifuge tubes
lysozyme solution
TENS solution
TE buffer
3M sodium acetate solution
95% ethanol
1 ml pipette
micropipettes
vortex (optional)
ice bath

1. Pour 6-8 ml of overnight culture of E.coli into a microcentrifuge tube and centrifuge for five minutes

2. After discarding the supernatant fluid, add .5 ml of lysozyme solution to the cell pellet and vortex or mix and let stand for five minutes.

3. Add .5 ml of TENS solution, mix and place tube on crushed ice bath for 15 minutes.

4. Add 0.5 ml of sodium acetate solution to neutralize the mixture and renature the plasmid DNA.

5. Centrifuge the solution for 10 minutes to pellet cell debris.

6. Transfer the supernatant fluid to a clean test tube and add 2 ml of 95% ethanol(which was in a freezer) and mix for 10 seconds and place on ice for 10 mins.

7. Centrifuge for 10 minutes to pellet the plasmid DNA

8. Discard the supernatant fluid and rinse the pellet twice with 95% ethanol.

9. Dry the pellet for 15 minutes in the air.

10. Add 40 ul of TE buffer and vortex/mix for 30 seconds. The solution should contain the plasmid.

11. Save the microcentrifuge tube of plasmid for experiment #5.

EXPERIMENT #4 EXTRACTION & PURIFICATION OF A PLASMID

Teacher's Guide:

Time Frame: two class periods of 50 minutes(if materials prepared ahead of time)

Target Group: 2nd-3rd year Biology Students

Preparation of Materials:

1. Flocculating Agent: 10% of Poly(ethylinemine) in distilled water

2. TENS solution: .4 grams of NaOH
   1.4 grams of SDS
   sodium dodecyl sulfate)
   100 ml of distilled water

3. TE Buffer(ph 8): .19 grams of Tris HCl
   .09 grams Tris
   .07 grams of EDTA
   dissolve in 200 ml in distilled water

4. Precipitating Agent: 60 ml of 5M potassium acetate
   11.5 ml of glacial acetic acid
   28.5 ml of distilled water

5. Isopropyl Alcohol: 100 ml of 90-100% isopropyl alcohol

6. Lysozyme Solution: .24 grams of Tris HCl
   .12 grams of Tris
   .34 grams EDTA
   1.71 grams of sucrose
   100 ml of distilled water

7.Sodium Acetate Solution(pH 5.2):
10 ml of 3M sodium acetate by
4.1 grams of sodium acetate
10 ml of distilled water
add 1.5 ml acetic acid to adjust
pH to 5.2

8. Ampicillin Stock: dissolving .5 gr of ampicillin
to 25 ml of distilled water, then
sterize by passing through .45
micron sterilizing filter

Sources of Materials:

Sigma Chemical CO.
P.O. Box 14508
St. Louis, MO. 63178-9916

Biochemical Stores
Biochemistry Building
Michigan State Univeristy
East Lansing, MI. 48824

Poly(ethyleimenine)#A3143
50 ml/$9.15
Millex Filters
#15900 filter .45 m $1.46
#15084 1/16 female luer $.21

Modern Biology
P.O. Box 97
Dayton, Indiana
47941-0097

Comments:

This lab is being refined. There are still some problems with the filter systems of the cheeese cloth and the millex filters. Finding the correct courseness of material to filter out the debris but not the plasmid is difficult.

References:

Anderson, John N., A Laboratory Course in Modern Biology, Purdue Univeristy, 1986.

High School Biology and Chemistry Research Manual, Workshop in Molecular Biology, Michigan State Universit 1989 and 1990.

Maniatis, T., E.T. Fritsch and J. Sambrook, Molecular Cloning: A laboratory Manual, Cold Spring Harbor Lab, Cold Spring Harbor, New York, 1982.

Smith, Richard G., Recombinant DNA Workstation, Loftstrand Labs Limited, Gaithersburg, Maryland,1989.

Weaver, Bob, "Plasmid Isolation", N.S.F. workshop paper Michigan State University, 1990.

EXPERIMENT #5 GEL ELECTROPHORESIS OF DNA FRAGMENTS

Background Information:

The DNA molecule is very large. With the use of restriction nuclease enzymes it can be cut in precise locations. The resultant fragments can be separated by the use of electrical current through a medium or substrate. Different molecular weight fragments of DNA will move through an agarose gel at different rates depending on charge and size of the fragment. The pUC18 plasmid that transformed the bacterium E. coli, can be reisolated.The reisolated DNA from experiment #4 can be visualized by gel electrophoresis.

In this lab, you will be using two different gel elctrophoresis procedures to isolate DNA fragments. First you will take the pUC18 plasmid that you isolated in Lab #4 and run a gel against a known pUC18 plasmid to isolate it and show its presence. Evidence of DNA (plasmid) will indicate your success in lab #4.

In the second part of the lab, you will use restriction enzymes to cut up a long strand of DNA called "lambda DNA". The lambda DNA contains 48,502 base pairs. The restriction enzymes will cut the DNA at specific sites. The two restriction enzymes you will use are EcoRI and BamH1. They will cut the DNA into six fragments. You will run the fragments in a gel against a well that contains "known size fragments of DNA" to compare the results.

Objective: To identify the plasmid(pUC18) removed in experiment #4 by the use of gel electrophoresis and to study the effects of the restriction enzymes, EcoR1 and BamH1, on phage lambda DNA and identify the restriction fragments.

Materials:

Electrophoresis Equipment
Agarose
Distilled water
Electrophoresis buffer
Methylene blue stain
Running dye mixture
phage DNA
restriction enzymes:EcoR1 and BamH1
water bath at 37 degrees
ice bath
micropipetes of (5 ul, 10 ul, and 20 ul)
sterile microcentrifuge tubes
Power source of 24 volts to 50 volts

Procedure:

Part 1 - pUC18 Plasmid Isolation by Electrophoreisis

1. Make three gels to use in electrophoresis by adding 1.2 grams of agarose to 100 ml of electrophoresis buffer. Bring to a SLIGHT boil in the microwave(it takes about 1 minute at high power). Make sure all the agarose has dissolved.

2. Let the agarose sit for two minutes to cool, then pour into trays with combs placed about 1 inch from one end. Allow to solidify.

3. Once the gel is set, remove the comb and the tape from the edges of the tray.Place the tray in the chamber for electrophoresis.

4. Cover the gel with electrophoresis buffer until it just covers the top of the gel.

5. Take the sample of possible plasmid from experiment #4 and mix 60 ul of glycerol with it.

6. Load well #1, #3, and #5 with a 20 ul sample.

7. Load a 20 ul sample of "known pUC18 plasmid" in wells #2 and #4. Load a 20 ul sample of the running dye in well #6.

8. Draw a diagram of the gel with the wells and the location of the various materials. HINT: cut one corner of the gel off as a reference point to finding the correct wells after staining.

9. Connect the positive lead of the power source to the electrode furthest from the wells. DNA is negatively charged and will migrate toward the positive charge. Connect the negative lead to the other electrode.

10. Turn on the power supply and watch for bubbles to form near the carbon rods. If the power supply is 24 volts it will take about two hours for the gel to completely separate the fragments or to"run". You can watch the progress of the separation by watching the colors separate from the running dye, when the first color gets 1 cm from the end of the gel - turn off the power supply.

11. After the gel has run, disconnect the power supply, take the gel out and measure the distances from the wells, the running dye colors moved in electrophoresis. Measure in millimeters from the well to the center of the color band(three color bands) and record. Draw a picture of the gel following electrophoresis

12. Place the gel in a shallow dish and cover the gel (flood) with 1% methylene blue stain(USE GLOVES) and let it stain for 15 minutes.

13. Wearing Gloves, remove the gel from the stain and rinse in distilled water for two minutes. Repeat the process four times. The stain can be saved and reused many times.

14. After the final rinse, wrap the gel in plastic wrap and refrigerate for 24 hours to develop the bands of DNA

15. NEXT DAY: measure the bands of dark blue stain that will be found in the lanes next to the wells. Record the measurements in mm. on the diagram you made of the running dye distances.

16. The data from this experiment will be graphed on semi-log paper to determine the size of each of the uncut DNA fragments. See teacher for further help.

Part 2: Restriction Enzyme Action on phage DNA

1. Label four sterile microcentrifuge tubes #1 - #4. with a waterproof marker.

2. Load each of the tubes with the following:
   #1 - 10 ul of predigested DNA,5 ul of glycerol
   #2 - 10 ul of EcoR1, 5 ul of glycerol
   #3 - 10 ul of EcoR1, 10 ul of BamH1, & 5 ul of glycerol
   #4 - 10 ul of BamH1, 5 ul of glycerol

3. Add 5 ul of lambda DNA to each tube except #1. Tap the tubes with your finger to mix.Incubate the tubes in a 37 degree water bath for 30 minutes.

4. Place one of the gels in the tray of the electrophoresis chamber and fill with electrophoresis solution to the top of the gel.

5. Load 15 ul samples into the wells as listed below:

Well Number	Tube Number
1	1(predigested DNA)
2	2(EcoR1)
3	3(EcoR1 & BamH1)
4	4(BamH1)
5	Running Dye(kit)
6	2(EcoR1)
7	3(EcoR1 & BamH1)
8	4(BamH1)

6. Run the gels similarly to Part 1

7. Remove the gels and measure the distances of the color bands of the running dyes and record. Draw a picture of the gel and label the wells and draw in distances of the bands.

8. Stain the gel similar to Part 1,steps 13 & 14

9. Store overnight in the refrigerator

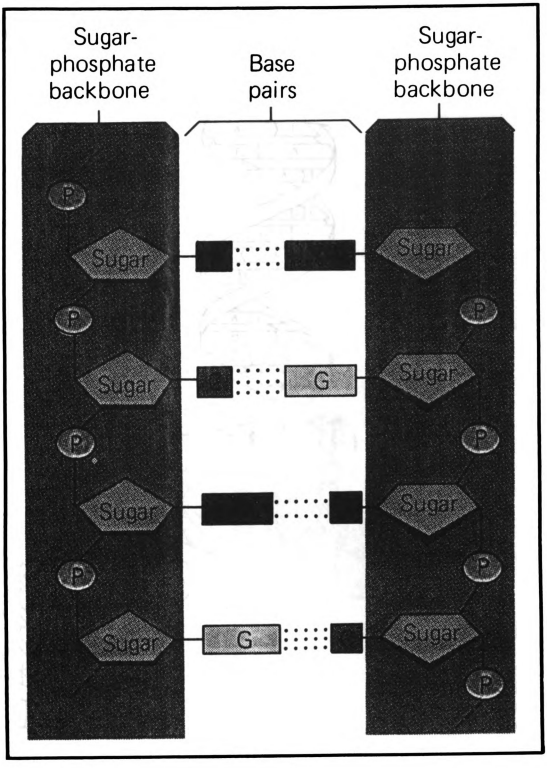
10. Measure from the wells the bands on dye found in ach well lane and record. Graph the data of the known predigested DNA on semi-log paper and calculate the sizes of fragments of pieces digested by EcoR1 and BamH1.

Results:

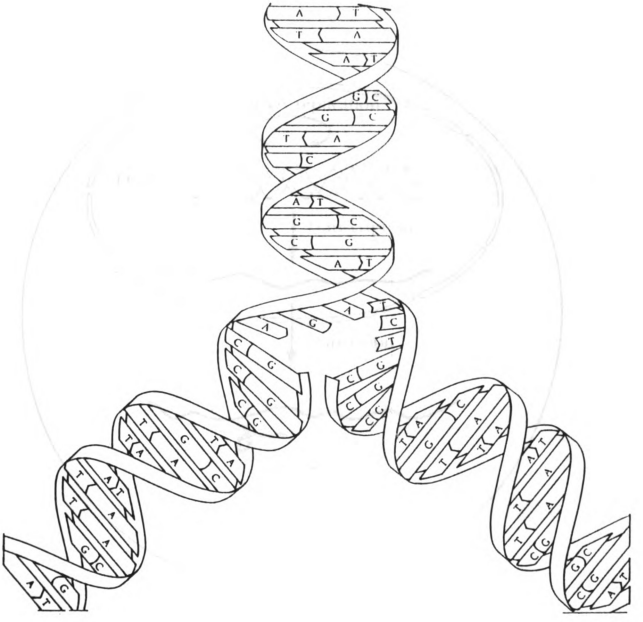
1. Graph the known fragment of DNA on the semi-log paper and then the fragments you cut with the two restriction enzymes to find the number of base pairs of each of the pieces of DNA.

APPENDIX B

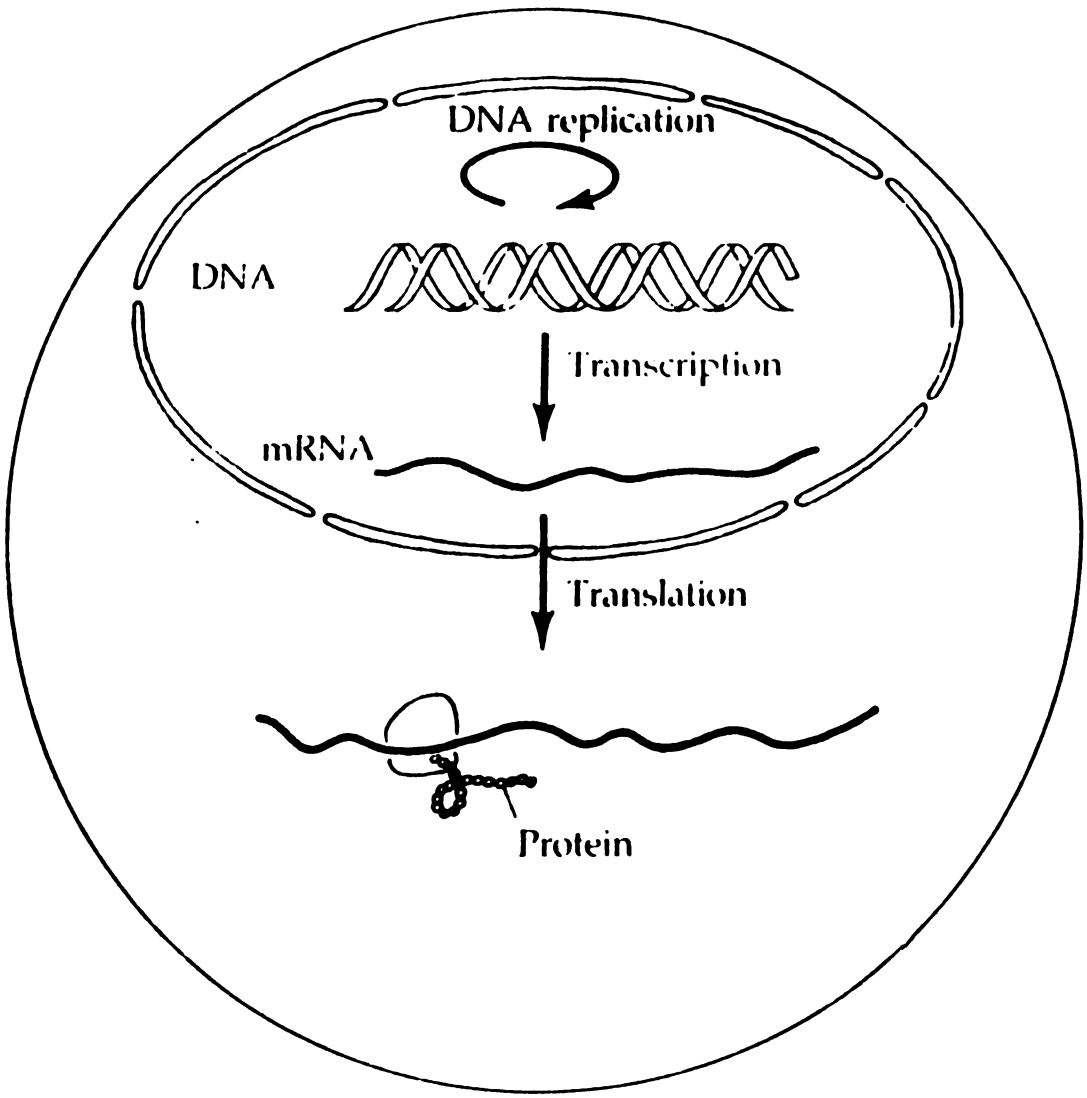
OVERLAYS USED IN UNIT



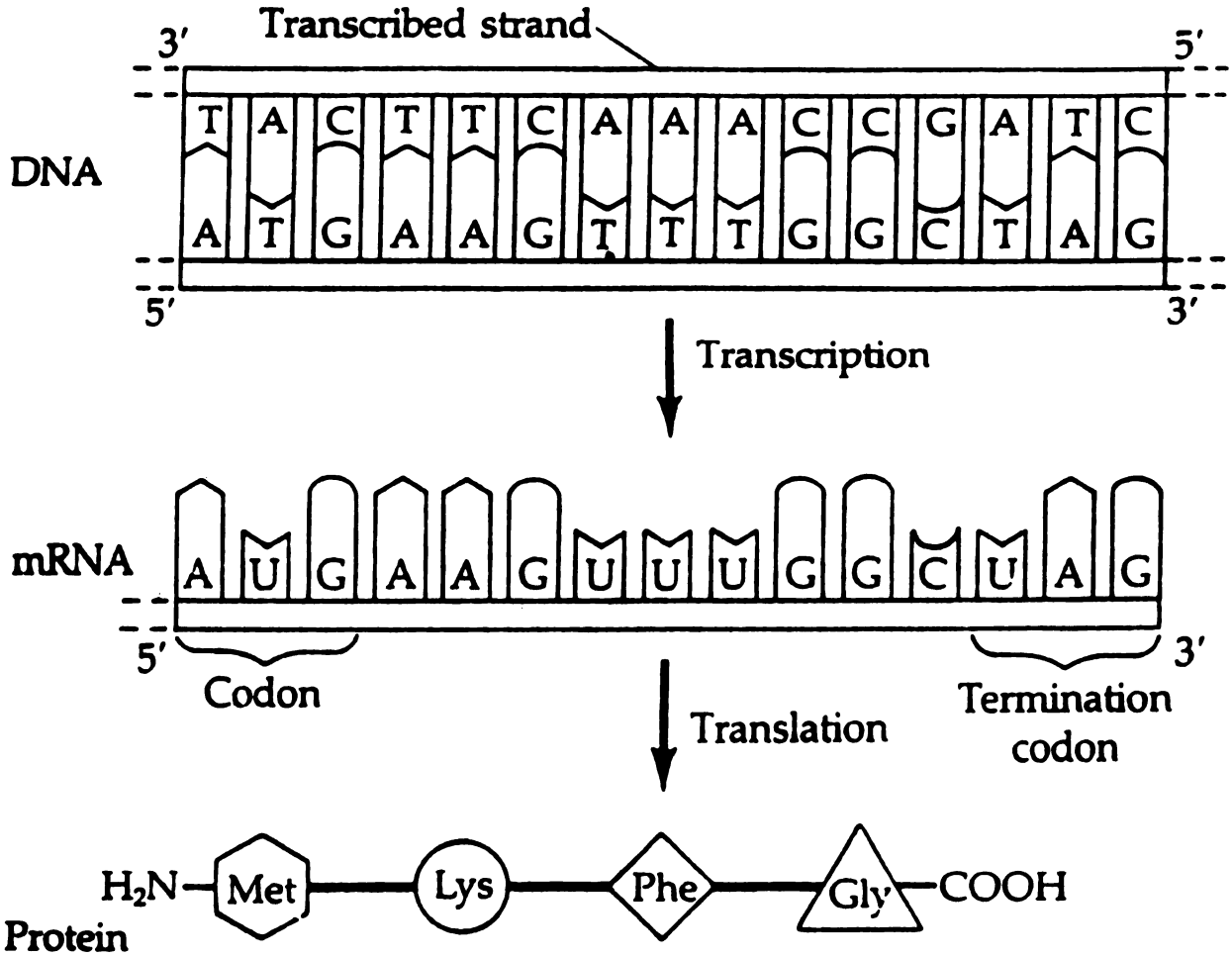
Semiconservative replication of DNA
Figure 15.9



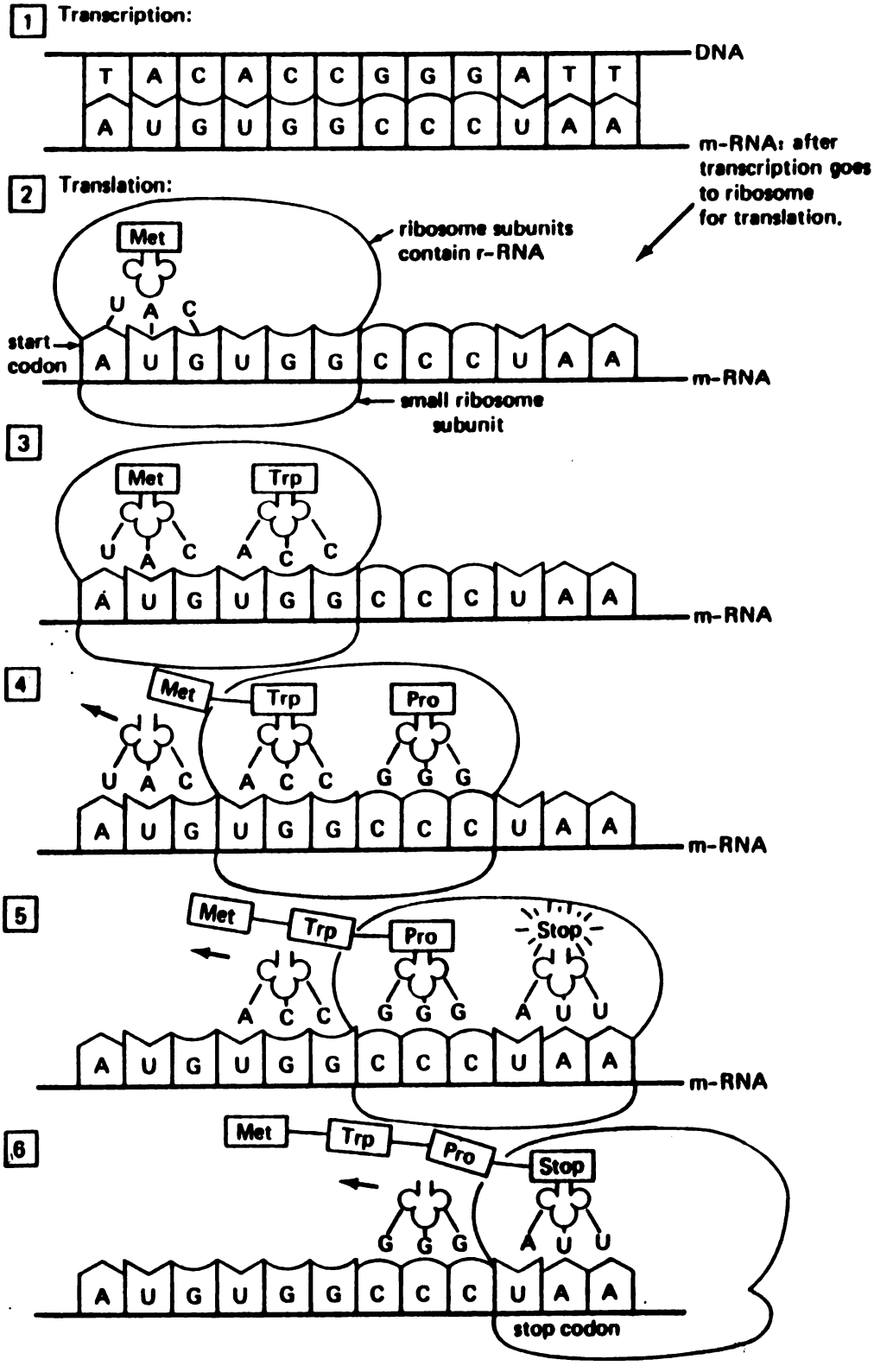
Central dogma of molecular biology (Figure 16.1)



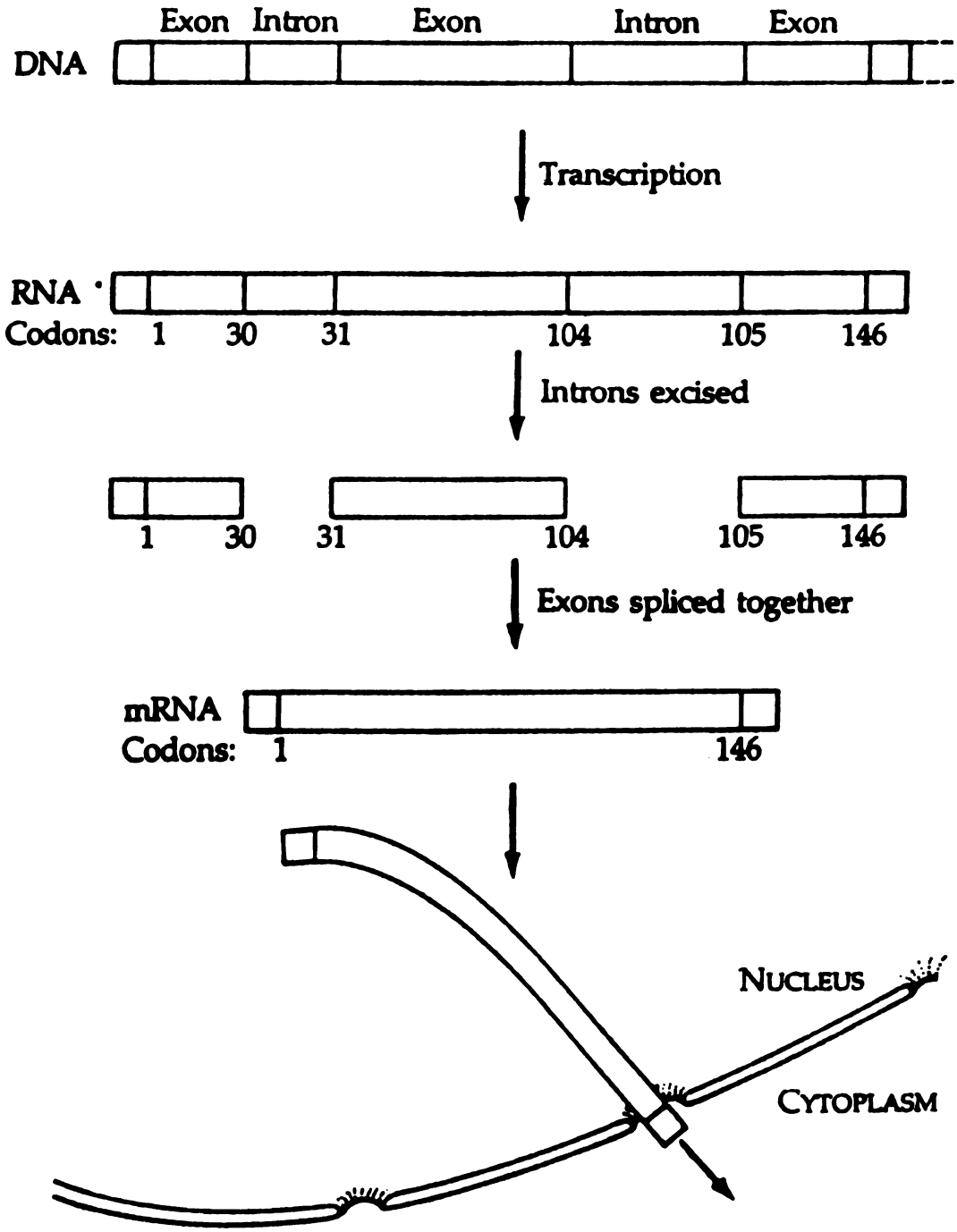
Transcription and translation of a DNA sequence
Figure 16.15

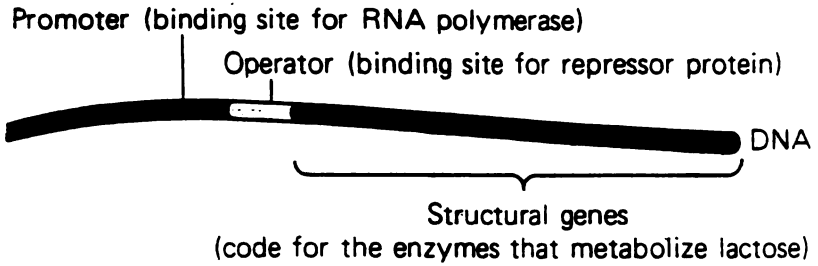


PROTEIN SYNTHESIS

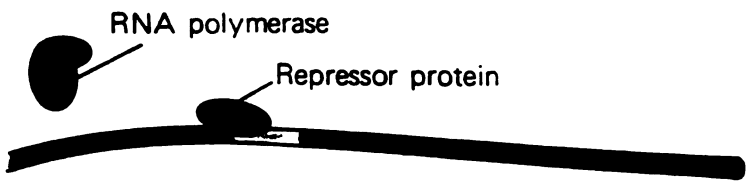


Introns in a eukaryotic gene
Figure 16.17

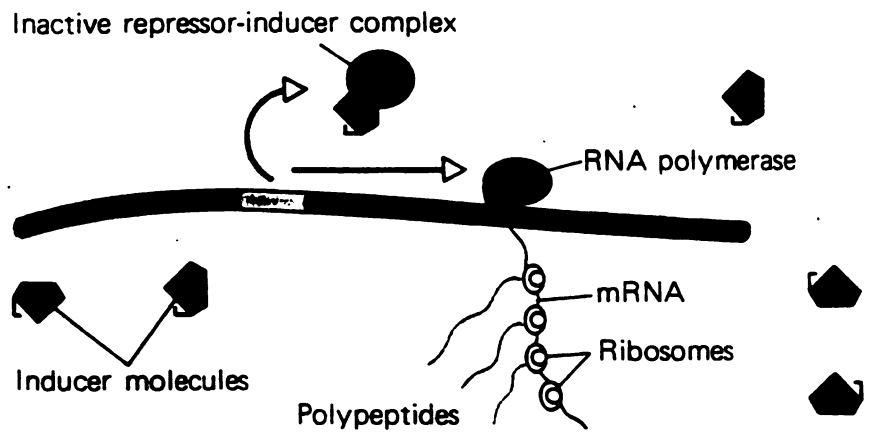




(a) The *lac* operon, a collection of genes that controls production of the enzymes of lactose metabolism



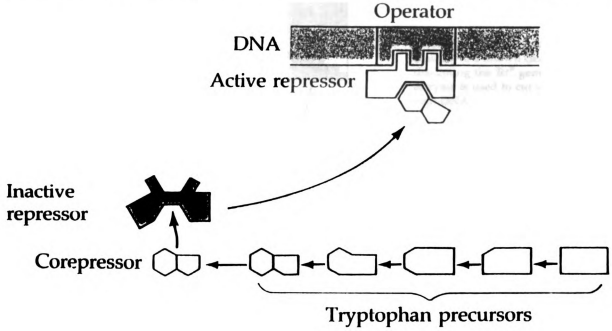
(b) In the absence of lactose, a repressor binds to the operator and prevents RNA polymerase from binding to the promoter



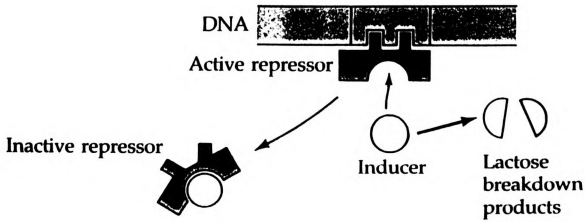
(c) In the presence of lactose, inducer molecules bind to the repressor and remove it from the operator. RNA polymerase can now bind to the promoter and transcribe the genes in the operon, producing mRNAs. Ribosomes immediately bind to the mRNAs and produce the polypeptides that metabolize lactose

Two types of negative control (Figure 17.22)

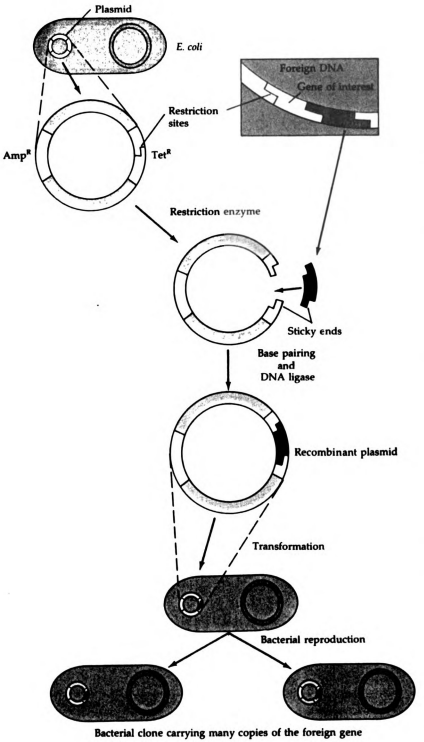
Example: *trp* operon — encodes enzymes for tryptophan synthesis



Example: *lac* operon — encodes enzymes for lactose breakdown



Cloning of a gene (Methods box, p. 401)



The plasmid is isolated from *E. coli*, and "foreign" DNA is isolated from other cells.

A restriction enzyme opens the plasmid at a known cleavage site, disrupting the Tet^R gene. The same enzyme is used to cut up the foreign DNA.

The sticky ends of the plasmid hydrogen-bond with the sticky ends of a restriction fragment from the foreign DNA. The two DNA molecules are joined covalently by DNA ligase.

The result is recombinant DNA, an *E. coli* plasmid carrying DNA from another source.

The recombinant plasmid is introduced into a bacterial cell by transformation. As the bacterium reproduces, so does the recombinant plasmid. The final result is a bacterial clone (colony) in which the foreign gene has been cloned. Colonies carrying recombinant plasmids can be identified by the fact that their cells are ampicillin-resistant (since they carry the Amp^R gene) but tetracycline-sensitive (since the Tet^R gene has been inactivated by the insertion of foreign DNA). Thus, the cells will grow on media containing ampicillin but not on media with tetracycline. More sophisticated methods must be used to identify the recombinant clones carrying the particular gene of interest, as discussed in the text.

APPENDIX C

OUTLINE FOR MOLECULAR BIOLOGY UNIT

OUTLINE FOR THE MOLECULAR BIOLOGY UNIT

TEXTBOOK: BIOLOGY: JOURNEY INTO LIFE
by Arms and Camp (1990)

Chapter 9: DNA and Genetic Information

I. Terminology: DNA - Chromosomes - genes - replication - proteins - DNA polymerase - amino acids - polymers - nucleotides

II. DNA Evidence as Genetic Material:
   A. Bacterial Transformation: Griffith(1928) virulent vs nonvirulent
   B. Bacteriophages(phages):Hershey/ Margarent Chase (1952)

III. Structure of DNA:
   A. Four experimental findings of the 1950's
   B. Franklin's work
   C. Watson and Crick work

EXPERIMENT #1 DNA ISOLATION

IV. DNA Replication:
   A. Template Hypothesis
   B. DNA polymerase
   C. Prokaryotes vs eukaryotes

EXPERIMENT #2 PROPERTIES OF DNA

V. DNA Repair
   A. Repair genes
   B. Mutations: mutagens-types and effects

VI. Structure of Eukaryotic Chromosomes
   A. Properties of Chromatin
   B. Chromosomal Proteins: jistones
   C. Nucleosomes (drawings)
   D. Genomes - Research
   E. Jumping Genes: McClintock's work(1940) transposable elements

Chapter 10: RNA & Protein Synthesis

I. Terminology: RNA - gene expression - structural genes - proteins - mRNA - tRNA - ATP - transcription - translation - operon gene

II. Overview of Protein Synthesis: felt board

transcription translation

DNA mRNA protein

III. Genetic Code:

A. four nucleotides: three letter "words" (codons) 64 different codes

B. Punctuation: effects on proteins

C. Codons: full stop/nonsense codons, degeneration frameshift mutations.

IV. Transcription of DNA into RNA:

A. RNA polymerase - termination sign - M-RNA role

B. Introns - intervening sequence

C. T-RNA role: aminoacyl attachment site; anticodon

V. Protein Synthesis:

A. Initialization: polypeptide chain formation (translocation), termination

B. Control of Protein Synthesis: eukaryotic vs prokaryotic cells; overview of the "Operon Theory" of Jacob/Monad

C. Operon Theory: structural genes, prometer, regulatory, repressor, inducers

D. HANDOUT: "Operon Theory"

E. Control in Eukaryotes: change in chromosomes, giant chromosomes, regulators

VI. Control of Gene Activity: differentiation, metamorphosis, and regeneration.

Chapter 11: New Genetics: Molecular Technology

I. Terminology: molecular biology, genetic engineering, hybrid, and hybridization

DRY LAB: PLASMID LAB (models of plasmid cloning)

II. Bacterial Restriction enzymes
    A. Techniques of Amplify

IV. Cloning of DNA
    A. clone - vector(plasmid)
    B. technique in bacteria/yeast restriction enzymes - sticky ends typical recombinant DNA experiment

EXPERIMENT #4 ISOLATION OF PLASMID DAN

V. Sequencing DNA
    A. Electrophoresis - procedure
    B. Human Geome Project

EXPERIMENT #5 GEL ELECTROPHORESIS

VI. Application of Genetic Technology
    A. Recombinant DNA: insulin, interferons, feline vaccine
    B. Reverse Genetics: identifying defective proteins such as M.S., Huntington's Disease, cystic fibrosis, Alzheimer's Disease
    C. Gene Transplants: improve crops disease resistance and nitrogen fixing ability; implant cell on the zygote(totipotent), and nuclear transplant of germ cells
    D. Cancer:
       Tumors-malignant(metastasize)
       Causes-mutagen
       Activation-onocogenes:translocation, retroviruses, AIDS, latent period
    E. Safety of Genetic Engineering

APPENDIX D

PRETEST & POST TEST

PRETEST ON GENETIC INFORMATION FORM: #201

INSTRUCTOR:_____ DATE:_____

CLASS:_____ STUDENT NAME:_____

DIRECTIONS: FOR EACH QUESTION, CIRCLE THE BEST ANSWER.

1) If the base pairing rules, i.e., A=T, G=C, are followed, then in a double helix of DNA

A. A=G, C=T. B. A + T/G + C = 1.
C. A + T = G + C. D. A + G = C + T.

2) _____bonds join the two strands of DNA together to form the double helix.

A. Hydrogen B. Covalent C. Ionic D. Polar Covalent

3) Consider the DNA sequence: -C C G A T G -. The complementary strand made after replication is

A. -C C G A T G-. B. -G G C T A C-.
C. -T T A G C A-. D. -T T A G C A-.

4) Translation is the process in which _____ is synthesized from _____.

A. DNA, RNA B. mRNA, the nucleolus
C. a polypeptide, mRNA D. tRNA, DNA

5) DNA contains _____ sugar and _____ while RNA contains sugar and ___.

A. ribose, uracil, deoxyribose, adenine
B. deoxyribose, adenine, ribose, thymine
C. ribose, quanine, riboose, quanine
D. deoxyribose, thymine, ribose, uracil

6) The termination of protein synthesis normally occurs

A. when the cell runs out of ATP
B. upon recognition of a stop codon.
C. upon recognition of an exon
D. when more than 10 ribosomes attach to the mRNA.

7) The operator of an operon

A. encodes information for the repressor protein.
B. is the binding site for the inducer.
C. is the binding site for the repressor.
D. controls only one structural gene.

8) The enzyme which joins two pieces of DNA together is called

A. restriction endonclease. B. ligase.
C. polymerase. D. Reverse transcriptase.

9) Cancer cells are characterized by

A. immortality. B. lack of contact inhibition.
C. uncontrolled growth. D. all of the above.

10) DNA can be introduced into the host cell by

A. viruses. B. plasmids. C. mutagens. D. a and b are correct.

11) Thirty percent of the bases in DNA extracted from prokaryotic cells is adenine. What percentage of cytosine is present in this DNA?

A. 10 B. 20 C. 30 D. 40

12) An actively dividing culture of E. COLI is grown in a medium containing radioactive thymine (*T). After all the thymine in the DNA is labeled the culture is transferred to a medium containing nonradioactive thymine (T). Samples are removed after one round of DNA replication. Which of the following sequences represents the DNA after one round of replication in the medium containing nonradioactive T?

A. *T *T A A G *T A
A A T T C A T

B. *T T A A G *T A
A A *T *T C A T

C. *T *T A A G *T A
A A *T *T C A *T

D. T T A A G T A
A A T T C A T

13) Which part(s) of the nucleotide is(are) essential for forming the structural backbone of the DNA molecule?

A. the base
B. the phosphate group
C. the base and the sugar
D. the phosphate group and the sugar

14) Consider the following double helix of DNA: A T C C G G G A
T A G G C G G T
Which of the following sequences represents a mutation of the original sequence?

A. A T C C G C C A
T A C G C G G A

B. A T C G C C C A
T A G C G G G T

C. T A G G C G G T
A T C C G G C A

D. all of the above

15) Which of the following sequences represents the hierarchial organization of genetic information?

A. gene - nucleotide - DNA - chromosome - genome
B. nucleotide - DNA - gene - chromosome - genome
C. genome - DNA - chromosome - gene - nucleotide
D. chromosome - gene - DNA - genome - nucleotide

16) Consider the following sequence of DNA: -C C G T A T G C T G C C-. The mRNA synthesized from this DNA is

A. -C C G T A T G C T G C C-
B. -C C G A U A G C A C G G-
C. -G G C A U A C G A C G G-
D. -G G C A T A C G A C G G-

17) Liver cells and brain cells isolated from the same mouse have identical quantities of DNA, but different amounts and types of mRNA's. On the basis of the above information, one might hypothesize that differentiation results from

A. the loss of chromosomes or genes.
B. differential gene activity.
C. the duplication of genes responsible for the differentiated state.
D. all of the above.

18) Consider the DNA sequence: -T A C G C C G A G C G C A C T-. The codons for select amino acids are:

codon	amino acid	codon	amino acid	codon	amino acid
GCG	alanine (ala)	GGC	glycine (gly)	CGG	arginine (arg)
AGU	serine (ser)	AUG	methionine (met)	CUC	leucine (leu)
CGU	arginine (arg)	UGC	cysteine (cys)	GUA	valine (val)
UCG	serine (ser)				

Which of the following sequences of amino acids would be encoded in the given DNA sequence?

A. met - arg - leu - ala
B. met - cys - gly - ser
C. ser - ala - leu - gly - val
D. ala - gly - ser - arg - val

19) In order for two DNA molecules to form a recombinant DNA, they must

A. be from the same organism.
B. be the same length.
C. have complementary sticky ends.
D. all of the above.

20) EcoRI, a restriction enzyme, recognizes the sequence GAATTC.
CTTAAG
Into how many fragments will EcoRI cut the double helix of DNA presented below?

DNA HELIX: C C G A T T C C G C T C G G A A T T C G A T T
G G C T A A G G C G A G C C T T A A G C T A A

A. 0 fragments B. 1 fragment C. 2 fragments D. 3 fragments

21) Recombinant DNA technology is possible because of

A. specific base pairing rules.
B. the universality of the genetic code.
C. restriction endonuclease.
D. all of the above.

DIRECTIONS: FOR EACH QUESTION, CIRCLE THE ONE BEST ANSWER.

22) TRUE or FALSE --- In DNA, the sugar and phosphate groups are covalent bonded together while the bases of the two strands are held by hydrogen bonds.

23) TRUE or FALSE --- All the DNA in eukaryotic cells contains information for making proteins.

24) TRUE or FALSE --- Griffith demonstrated the phenomenon of bacterial transformation.

25) TRUE or FALSE --- Introns contain the information for making a poly peptide.

26) TRUE or FALSE --- The genetic code is degenerate, almost universal, and lacks punctuation.

27) TRUE or FALSE --- Only DNA from the same or similar organisms can be combined to form a hybrid molecule.

28) TRUE or FALSE --- Restriction enzymes randomly cut DNA molecules.

29) TRUE or FALSE --- An oncogene functions in normal growth and embryogenesis, but has the potential to cause cancer.

30) TRUE or FALSE --- Metastasis is a characteristic of malignant cells.

DIRECTIONS: FOR EACH QUESTION, WRITE YOUR ANSWER ON THE LINE PROVIDED.

31) Which of the following scientists (Watson and Crick, Franklin, McClintock, Griffith, Chargaff) matches with his/her discovery or contribution to genetics: discovered jumping genes.

---

32) Which of the following scientists (Watson and Crick, Franklin, McClintok, Griffith, Chargaff) matches with his/her discovery or contribution to genetics: determined the helical structure of DNA.

---

33) Which of the following scientists (Watson and Crick, Franklin, McClintok, Griffith, Chargaff) matches with his/her discovery or contribution to genetics: discovered that the amount of A = T and G = C in DNA.

---

34) Which of the following scientists (Watson and Crick, Franklin, McClintok, Griffith, Chargaff) matches with his/her discovery or contribution to genetics: provided evidence from X-ray diffraction for the periodicity in DNA.

---

35) Which of the following scientists (Watson and Crick, Franklin, McClintok, Griffith, Chargaff) matches with his/her discovery or contribution to genetics: demonstrated bacterial transformation.

---

36) Which of the following terms (replication, acylation, translation, translocation, transcription) matches the definition: movement of ribosome along mRNA.

---

37) Which of the following terms (replication, acylation, translation, translocation, transcription) matches the definition: synthesis of RNA from DNA.

---

38) Which of the following terms (replication, acylation, translation, translocation, transcription) matches the definition: synthesis of DNA from DNA.

---

39)Which of the following terms (replication, acylation, translation, translocation, transcription) matches the definition: attachment of amino acid to tRNA.

---

40) Which of the following terms (replication, acylation, translation, translocation, transcription) matches the definition: conversion of language of nucleotides to language of amino acids.

---

41) Which of the following terms (vector, zygote, oncogene, clone, plasmid) matches the definition: group of genetically identical organisms or cells.

---

42) Which of the following terms (vector, zygote, oncogene, clone, plasmid) matches the definition: group of genetically identical organisms or cells.

---

43) Which of the following terms (vector, zygote, oncogene, clone, plasmid) matches the definition: fertilized egg.

---

44) Which of the following terms (vector, zygote, oncogene, clone, plasmid) matches the definition: small circular DNA molecule in bacterial cell.

---

45) Which of the following terms (vector, zygote, oncogene, clone, plasmid) matches the definition: carrier of DNA.

ANSWER KEY FOR: PRETEST ON GENETIC INFORMATION FORM:#201

INSTRUCTOR: _____DATE: _____

CLASS:_____

If you are using alternate forms, be sure that the form # on this answer key matches the student's answer sheet form #.

1) - - C - -
2) A - - - -
3) - B - - -
4) - - C - -
5) - - - D -
6) - B - - -
7) - - C - -
8) - B - - -
9) - - - D -
10) - - - D -
11) - B - - -
12) A - - - -
13) - - - D -
14) - - - D -
15) - B - - -
16) A - - - -
17) - B - - -
18) A - - - -
19) - - C - -
20) - B - - -
21) - - - D -
22) T -
23) - F
24) T -
25) - F
26) T -
27) - F
28) - F
29) T -
30) T -

MATCHING (SHORT-ANSWER) QUESTIONS:

31) McClintock
32) Watson and Crick
33) Chargaff
34) Franklin
35) Griffith
36) translocation
37) transcription
38) replication
39) acylation
40) translation
41) oncogenes
42) clone
43) zygote
44) plasmid
45) vector

A.P.BIOLOGY CH.9-11 FORM: #201

INSTRUCTOR:_____ DATE:_____

CLASS:_____ STUDENT NAME:_____

DIRECTIONS: FOR EACH QUESTION, CIRCLE THE BEST ANSWER.

1) If the base pairing rules, i.e., A=T, G=C, are followed, then in a double helix of DNA

A. A=G, C=T. B. A + T/G + C = 1.
C. A + T = G + C. D. A + G = C + T.

2) _____ bonds join the two strands of DNA together to form the double helix.

A. Hydrogen B. Covalent C. Ionic D. Polar Covalent

3) Consider the DNA sequence: -C C G A T G -. The complementary strand made after replication is

A. -C C G A T G-. B. -G G C T A C-.
C. -T T A G C A-. D. -T T A G C A-.

4) Translation is the process in which _____ is synthesized from _____.

A. DNA, RNA B. mRNA, the nucleolus
C. a polypeptide, mRNA D. tRNA, DNA

5) DNA contains _____ sugar and _____ while RNA contains sugar and ___.

A. ribose, uracil, deoxyribose, adenine
B. deoxyribose, adenine, ribose, thymine
C. ribose, quanine, riboose, quanine
D. deoxyribose, thymine, ribose, uracil

6) The termination of protein synthesis normally occurs

A. when the cell runs out of ATP
B. upon recognition of a stop codon.
C. upon recognition of an exon
D. when more than 10 ribosomes attach to the mRNA.

7) The operator of an operon

A. encodes information for the repressor protein.
B. is the binding site for the inducer.
C. is the binding site for the repressor.
D. controls only one structural gene.

8) The enzyme which joins two pieces of DNA together is called

A. restriction endonclease. B. ligase.
C. polymerase. D. Reverse transcriptase.

9) Cancer cells are characterized by

A. immortality. B. lack of contact inhibition.
C. uncontrolled growth. D. all of the above.

10) DNA can be introduced into the host cell by

A. viruses. B. plasmids. C. mutagens. D. a and b are correct.

11) Thirty percent of the bases in DNA extracted from prokaryotic cells is adenine. What percentage of cytosine is present in this DNA?

A. 10 B. 20 C. 30 D. 40

12) An actively dividing culture of E. COLI is grown in a medium containing radioactive thymine (*T). After all the thymine in the DNA is labeled the culture is transferred to a medium containing nonradioactive thymine (T). Samples are removed after one round of DNA replication. Which of the following sequences represents the DNA after one round of replication in the medium containing nonradioactive T?

A. *T *T A A G *T A
A A T T C A T
B. *T T A A G *T A
A A *T *T C A T
C. *T *T A A G *T A
A A *T *T C A *T
D. T T A A G T A
A A T T C A T

13) Which part(s) of the nucleotide is(are) essential for forming the structural backbone of the DNA molecule?

A. the base
B. the phosphate group
C. the base and the sugar
D. the phosphate group and the sugar

14) Consider the following double helix of DNA: A T C C G G G A
T A G G C G G T

Which of the following sequences represents a mutation of the original sequence?

A. A T C C G C C A
T A C G C G G A
B. A T C G C C C A
T A G C G G G T
C. T A G G C G G T
A T C C G G C A
D. all of the above

15) Which of the following sequences represents the hierarchial organization of genetic information?

A. gene - nucleotide - DNA - chromosome - genome
B. nucleotide - DNA - gene - chromosome - genome
C. genome - DNA - chromosome - gene - nucleotide
D. chromosome - gene - DNA - genome - nucleotide

16) Consider the following sequence of DNA: -C C G T A T G C T G C C-. The mRNA synthesized from this DNA is

A. -C C G T A T G C T G C C-
B. -C C G A U A G C A C G G-
C. -G G C A U A C G A C G G-
D. -G G C A T A C G A C G G-

17) Liver cells and brain cells isolated from the same mouse have identical quantities of DNA, but different amounts and types of mRNA's. On the basis of the above information, one might hypothesize that differentiation results from

A. the loss of chromosomes or genes.
B. differential gene activity.
C. the duplication of genes responsible for the differentiated state.
D. all of the above.

18) Consider the DNA sequence: -T A C G C C G A G C G C A C T-. The codons for select amino acids are:

codon	amino acid	codon	amino acid	codon	amino acid
GCG	alanine (ala)	GGC	glycine (gly)	CGG	arginine (arg)
AGU	serine (ser)	AUG	methionine (met)	CUC	leucine (leu)
CGU	arginine (arg)	UGC	cysteine (cys)	GUA	valine (val)
UCG	serine (ser)				

Which of the following sequences of amino acids would be encoded in the given DNA sequence?

A. met - arg - leu - ala
B. met - cys - gly - ser
C. ser - ala - leu - gly - val
D. ala - gly - ser - arg - val

19) In order for two DNA molecules to form a recombinant DNA, they must

A. be from the same organism.
B. be the same length.
C. have complementary sticky ends.
D. all of the above.

20) EcoRI, a restriction enzyme, recognizes the sequence GAATTC.
CTTAAG
Into how many fragments will EcoRI cut the double helix of DNA presented below?

DNA HELIX: C C G A T T C C G C T C G G A A T T C G A T T
G G C T A A G G C G A G C C T T A A G C T A A

A. 0 fragments B. 1 fragment C. 2 fragments D. 3 fragments

21) Recombinant DNA technology is possible because of

A. specific base pairing rules.
B. the universality of the genetic code.
C. restriction endonuclease.
D. all of the above.

DIRECTIONS: FOR EACH QUESTION, CIRCLE THE ONE BEST ANSWER.

22) TRUE or FALSE --- In DNA, the sugar and phosphate groups are covalent bonded together while the bases of the two strands are held by hydrogen bonds.

23) TRUE or FALSE --- All the DNA in eukaryotic cells contains information for making proteins.

24) TRUE or FALSE --- Griffith demonstrated the phenomenon of bacterial transformation.

25) TRUE or FALSE --- Introns contain the information for making a poly peptide.

26) TRUE or FALSE --- The genetic code is degenerate, almost universal, and lacks punctuation.

27) TRUE or FALSE --- Only DNA from the same or similar organisms can be combined to form a hybrid molecule.

28) TRUE or FALSE --- Restriction enzymes randomly cut DNA molecules.

29) TRUE or FALSE --- An oncogene functions in normal growth and embryogenesis, but has the potential to cause cancer.

30) TRUE or FALSE --- Metastasis is a characteristic of malignant cells.

DIRECTIONS: FOR EACH QUESTION, WRITE YOUR ANSWER ON THE LINE PROVIDED.

31) Which of the following scientists (Watson and Crick, Franklin, McClintock, Griffith, Chargaff) matches with his/her discovery or contribution to genetics: discovered jumping genes.

---

32) Which of the following scientists (Watson and Crick, Franklin, McClintok, Griffith, Chargaff) matches with his/her discovery or contribution to genetics: determined the helical structure of DNA.

---

33) Which of the following scientists (Watson and Crick, Franklin, McClintok, Griffith, Chargaff) matches with his/her discovery or contribution to genetics: discovered that the amount of A = T and G = C in DNA.

---

34) Which of the following scientists (Watson and Crick, Franklin, McClintok, Griffith, Chargaff) matches with his/her discovery or contribution to genetics: provided evidence from X-ray diffraction for the periodicity in DNA.

---

35) Which of the following scientists (Watson and Crick, Franklin, McClintok, Griffith, Chargaff) matches with his/her discovery or contribution to genetics: demonstrated bacterial transformation.

---

36) Which of the following terms (replication, acylation, translation, translocation, transcription) matches the definition: movement of ribosome along mRNA.

---

37) Which of the following terms (replication, acylation, translation, translocation, transcription) matches the definition: synthesis of RNA from DNA.

---

38) Which of the following terms (replication, acylation, translation, translocation, transcription) matches the definition: synthesis of DNA from DNA.

---

39) Which of the following terms (replication, acylation, translation, translocation, transcription) matches the definition: attachment of amino acid to tRNA.

---

40) Which of the following terms (replication, acylation, translation, translocation, transcription) matches the definition: conversion of language of nucleotides to language of amino acids.

---

41) Which of the following terms (vector, zygote, oncogene, clone, plasmid) matches the definition: group of genetically identical organisms or cells.

---

42) Which of the following terms (vector, zygote, oncogene, clone, plasmid) matches the definition: group of genetically identical organisms or cells.

---

43) Which of the following terms (vector, zygote, oncogene, clone, plasmid) matches the definition: fertilized egg.

---

44) Which of the following terms (vector, zygote, oncogene, clone, plasmid) matches the definition: small circular DNA molecule in bacterial cell.

---

45) Which of the following terms (vector, zygote, oncogene, clone, plasmid) matches the definition: carrier of DNA.

ANSWER KEY FOR: A.P.BIOLOGY CH.9-11 FORM:#201

INSTRUCTOR: _____ DATE:_____

CLASS:_____

If you are using alternate forms, be sure that the form # on this answer key matches the student's answer sheet form #.

1) - - C - -
2) A - - - -
3) - B - - -
4) - - C - -
5) - - - D -
6) - B - - -
7) - - C - -
8) - B - - -
9) - - - D -
10) - - - D -
11) - B - - -
12) A - - - -
13) - - - D -
14) - - - D -
15) - B - - -
16) A - - - -
17) - B - - -
18) A - - - -
19) - - C - -
20) - B - - -
21) - - - D -
22) T -
23) - F
24) T -
25) - F
26) T -
27) - F
28) - F
29) T -
30) T -

MATCHING (SHORT-ANSWER) QUESTIONS:

31) McClintock
32) Watson and Crick
33) Chargaff
34) Franklin
35) Griffith
36) translocation
37) transcription
38) replication
39) acylation
40) translation
41) oncogenes
42) clone
43) zygote
44) plasmid
45) vector

APPENDIX E

FELTBOARD DEMONSTRATION

FELTBOARD DEMONSTRATION

I do not have the felt board presentation in a form that can be put down on paper. It consists of a white piece of felt approximately 3' by 3' on a stiff piece of cardboard. I cut out models of the parts needed to show transcription and translocation from colored felt, to indicate DNA, T-RNA, and M-RNA. The felt DNA shows two triplets and their nucleotides.

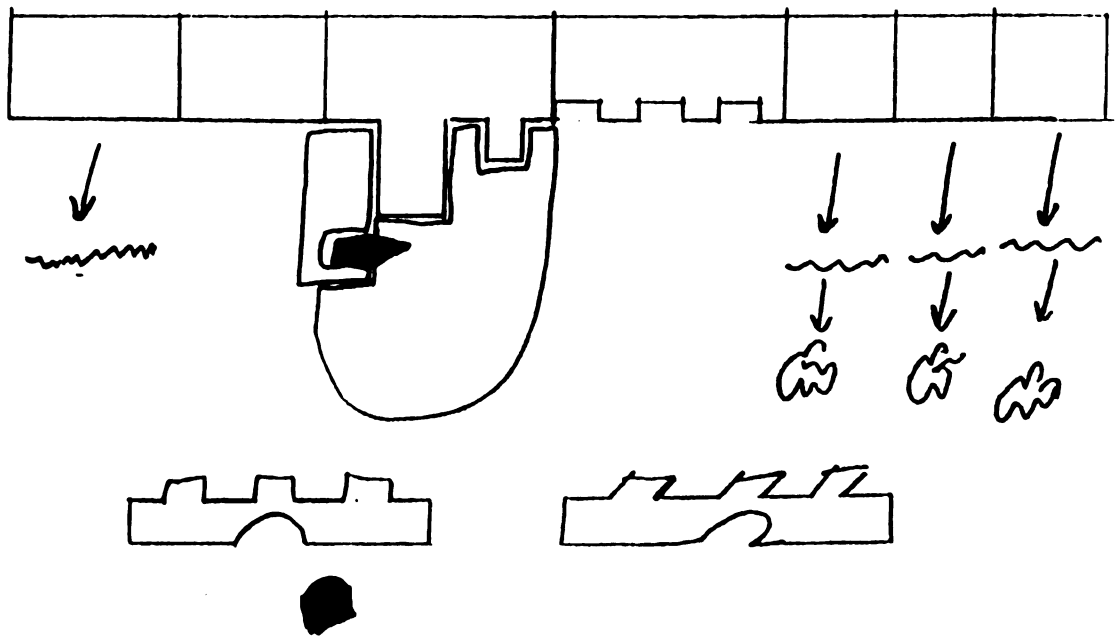
With different colored felt I made two small amino acid molecules and a large ribosomes model. By the cuts made on the T-RNA, they would only properly fit one type of amino acid. The T-RNA's also were matched to bind with one specific M-RNA codon.

With these simple moveable pieces of felt, it was possible to show both transcription and translocation. It was also possible to discuss the effects of slight changes in the DNA or M-RNA and how that might effect the protein product.

The felt board demonstration is simple, colorful, and effective way to review the concepts of protein synthesis with the students. They seem to enjoy the different type of approach I use here.

APPENDIX F

HANDOUTS USED IN THE UNIT



APPENDIX G

UNIT TESTS

A.P. BIOLOGY CHAPTER 9&10 FORM:#201

INSTRUCTOR: _____ DATE:_____

CLASS:_____ STUDENT NAME:_____

DIRECTIONS: FOR EACH QUESTION, CIRCLE THE ONE BEST ANSWER.

1) If the base pairing rules, i.e., A=T, G=C, are followed, then in double helix of DNA

A. A=G, C+T. B. A + T/G + C = 1
C. A + T = G + C. D. A + G = C + T.

2) Chargraff determined that

A. a gamete has half the DNA that is present in a liver cell from the same organism.
B. A = T and G = C
C. DNA is the genetic material.
D. DNA isolated from virulent bacteria could transform cells.

3) The enzyme responsible for DNA replication and proofreading is DNA

A. replicase. B. polymerase. C. synthetase. D. ligase.

4) _____ bonds that join the two strands of DNA together to form the double helix.

A. Hydrogen B. Covalent C. Ionic D. Polar covalent

5) This scientist's X-ray diffraction studies of DNA crystals provided important information regarding the structure of DNA. Who was the scientist?

A. Crick B. McClintok C. Franklin D. Hershey

6) All of the following are features of DNA isolated from eukaryotic cells except

A. organization into circular chromosomes.
B. association with histones.
C. organization into nucleosomes.
D. capability of mutation.

7) Mutations

A. cannot be passed to an organism's offspring.
B. always cause detrimental effects.
C. provide the raw material for evolution.
D. always result in deletions of nucleotides.

8) Consider the DNA sequence: - C C G A T G-. The complementary strand made after replication is

A. -C C G A T G-.
B. -G G C T A C-.
C. -T T A G C A-.
D. -T T A G C A-.

9) Which of the following events occurs first in the replication of DNA?

A. breaking of covalent binds between the nitrogenous bases
B. unwinding of the helix
C. breaking of the hydrogen bonds between the sugar and phosphate
D. addition of complementary nucleotides to one end of the DNA strand

10) Translation is the process in which _____ is synthesized from _____.

A. DNA, RNA
B. mRNA, the nucleolus
C. a polypeptide, mRNA
D. tRNA, DNA

11) For each amino acid there

A. is only one codon and tRNA.
B. can be more than one codon and tRNA.
C. are two codons, but only one tRNA.
D. are two tRNA molecules, but one codon.

12) In the process of acylation a(n) _____ is attached to a(n) _____ at its_ _____ site.

A. tRNA, mRNA, peptidyl
B. amino acid, tRNA, anticodon
C. codon, anticodon, aminoacyl
D. amino acid, tRNA, aminoacyl

13) DNA contains _____ sugar and _____ while RNA contains sugar and _____.

A. ribose, uracil, deoxyribose, adenine
B. deoxyribose, adrine, ribose, thymine
C. ribose, guanine, ribose, quanine
D. deoxyribose, thymine, ribose, uracil

14) The termination of protein synthesis normally occurs

A. when the cell runs out of ATP.
B. upon recognition of stop codon.
C. upon recognition of an exon.
D. when more than 10 ribosomes attach to the mRNA.

15) All of the following are directly required for protein synthesis except

A. GTP. B. ribosomes. C. tRNA. D. DNA.

16) All of the following are associated with increased transcription except

A. formation of lampbrush chromosomes.
B. binding of activator regulatory protein to the operator.
D. puffing of polytene chromosomes.

17) The operator of an operon

A. encodes information for the repressor protein.
B. is the binding site for the inducer.
C. is the binding site for the repressor.
D. controls only one structural gene.

18) Which of the following statements about genetic regulatory mechanisms is eukaryotic cells is true?

A. Genes are organized into operons whose activity is controlled by regulatory proteins.
B. Gene regulation occurs primarily at the level of translation.
C. Gene activation involves a change in the structure of the chromosome.
D. Histones play no role in the control of gene activity.

19) When a cell becomes differentiated,

A. gene no longer needed are lost.
B. genes responsible for the differentiated state are duplicated.
C. certain genes become inactivated.
D. all the chromatin becomes decondensed.

20) Thirty percent of the bases in DNA extracted from prokaryotic cells is adenine. What percentage of cytosine is present in this DNA?

A. 10 B. 20 C. 30 D. 40

21) For a double helix of DNA, which of the following statements is true?

A. A = G, C = T B. A = C, G = T C. A+T = G+C D. A = T, G = C

22) An actively dividing culture of E. COLI is grown in a medium containing radioactive thymine (*T). After all the thymine in the DNA is labeled the culture is transferred to a medium containing nonradioactive thymine (T). Samples are removed after one round of DNA replication. Which of the following sequences represents the DNA after one round of replication in the medium containing nonradioactive T?

A.	*T	*T	A	A	G	*T	A
	A	A	T	T	C	A	T
B.	*T	T	A	A	G	*T	A
	A	A	*T	*T	C	A	T
C.	*T	*T	A	A	G	*T	A
	A	A	*T	*T	C	A	*T
D.	T	T	A	A	G	T	A
	A	A	T	T	C	A	T

23) Which part(s) of a nucleotide is(are) essential for forming the structural backbone of the DNA molecule?

A. the base
B. the phosphate group
C. the base and the sugar
D. the phosphate group and the sugar

24) Which of the following criteria could you use to distinguish a prokaryotic from a eukaryotic chromosome?

A. shape
B. presence of protein
C. method of replication
D. all of the above

25) Consider the following double helix of DNA:

A T C C G G G A
T A G G C G G T.

Which of the following sequences represents a mutation of the original sequence?

A. A T C C G C C A
T A C G C G G A
B. A T C G C C C A
T A G C G G G T
C. T A G G C G G T
A T C C G G C A
D. all of the above

26) Which of the following sequences represents the hierarchial organization of the genetic information?

A. gene - nucleotide - DNA - chromosome - genome
B. nucleotide - DNA - gene - chromosome - genome
C. genome - DNA - chromosome - gene - nucleotide
D. chromosome - gene - DNA - genome - nucleotide

27) A strand of DNA has the sequence: A C *G C C T C A A G. The nucleotide marked by the (*) is mutated to a C. Which of the following sequences represents a DNA replicated from the mutated strand?

A. A C C C C T C A A G
B. T G G G G A G T T C
C. T G C G G A G T T C
D. G T A T T C T G G A

28) What is the minimum number of tRNA molecules required to produce a polypeptide containing 50 amino acids but only 15 different kinds of amino acids?

A. 15 B. 20 C. 35 D. 50

29) Consider the following sequence of DNA: -C C G T A T G C T G C C -. The mRNA synthesized form this DNA is

A. -C C G T A T G C T G C C - B. - C C G A U A G C A C G G -
C. - G G C A U A C G A C G G - D. - G G C A T A C G A C G G -

30) The type of bond between the anticodon and the codon, or between the codon and its triplet is a

A. hydrogen bond. B. covalent bond.
C. peptide bond. D. ionic bond.

31) Liver cells and brain cells isolated from the same mouse have identical quantities of DNA, but different amounts and types of mRNA's. On the basis of the above information, one might hypothesize that differentiation results from

A. the loss of chromosomes or genes.
B. differential gene activity.
C. the duplication of the genes responsible for the differentiated state.
D. all of the above.

32) Bacterial cells are grown for two hours in a medium containing lactose as the sole source of sugar, and then transferred into a medium with glucose as the sole source of carbohydrate. Which of the following most likely occurs after the cells are transferred to the glucose medium?

A. The structural genes in the lac operon continue to be transcribed.
B. A repressor protein binds to the prometer of the lac operon.
C. RNA polymerase no longer binds to the prometer of the lac operon.
D. The repressor protein inactivates RNA polymerase by binding to it.

33) Consider the DNA sequence: -T A C G C C G A G C G C A C T -. The codons for select amino acids are:

codon	amino acid	codon	amino acid	codon	amino acid
GCG	alanine (ala)	GGC	glycine (gly)	CGG	arginine (arg)
AGU	serine (ser)	AUG	methionine (met)	CUC	leucine (leu)
CGU	arginine (arg)	UGC	cysteine (cys)	GUA	valine (val)

Which of the following sequences of amino acids would be encoded in the given DNA sequence?

A. met - arg - leu - ala
B. met - cys - gly - ser
C. ser - ala - leu - gly - val
D. ala - gly - ser - srg - val

DIRECTIONS: FOR EACH QUESTION, CIRCLE THE ONE BEST ANSWER.

34) TRUE or FALSE --- Immediately after replication a cell has four times the amount of DNA found in a gamete from the same organism.

35) TRUE or FALSE --- The DNA from all organisms has the same amount of guanine and adenine.

36) TRUE or FALSE --- DNA replication represents a series of exergonic reactions.

37) TRUE or FALSE --- In DNA, the sugar and phosphate groups are covalently bonded together while the bases of the two strands are held by hydrogen bonds.

38) TRUE or FALSE --- DNA polymerase's sole function is to proofread and repair damage caused by mutagens.

39) TRUE or FALSE --- All the DNA in eukaryotic cells contains information for making proteins.

40) TRUE or FALSE --- Griffith demonstrated the phenomenon of bacterial transformation.

41) TRUE or FALSE --- Chromatin contains roughly equal amounts of DNA and histone and nonhistone proteins.

42) TRUE or FALSE --- Jumping genes are found only in prokayotes.

43) TRUE or FALSE --- In RNA the Ratio of the purine, adenine, to the pyrimidine, thymine is 1.

44) TRUE or FALSE --- Introns contain the information for making a polypeptide.

45) TRUE or FALSE --- The genetic code is degenerate, almost universal, and lacks punctuation.

46) TRUE or FALSE --- Eukaryotic mRNA molecules that are translated always contain the same number of nucleotides as the DNA from which they were transcribed.

47) TRUE or FALSE --- In translation the anticodon are covalently bonded together.

48) TRUE or FALSE --- A cell actively involved in protein synthesis would have very few ribosomes compared to one not engaged in protein synthesis.

49) TRUE or FALSE --- Uncoiled or decondensed chromatin is a sign of gene activity.

50) TRUE or FALSE --- In eukaryotes mRNA is translated as soon as it is transcribed.

DIRECTIONS: FOR EACH QUESTION, WRITE YOUR ANSWER ON THE LINE PROVIDED.

51) Which of the following scientists (Watson and Crick, Franklin, McClintock, Griffith, Chargaff) matches with his/her discovery or contribution to genetics: discovering jumping genes.

---

52) Which of the following scientists (Watson and Crick, Franklin, McClintock, Griffith, Chargaff) matches with his/her discovery or contribution to genetics: determined the helical structure of DNA.

---

53) Which of the following scientists (Watson and Crick, Franklin, McClintock, Griffith, Chargaff) matches with his/her discovery or contribution to genetics: discovered that the amount of A = T and G = C in DNA.

---

54) Which of the following scientists (Watson and Crick, Franklin, McClintock, Griffith, Chargaff) matches with his/her discovery or contribution to genetics: provided evidence from X-ray diffraction for the periodicity in DNA.

---

55) Which of the following scientists (Watson and Crick, Franklin, McClintock, Griffith, Chargaff) matches with his/her discovery or contribution to genetics: demonstrated bacterial transformation.

---

56) Which of the following terms (bacteriophage, thymine, guanine, nucleosome, nucleotide) matches the definition: composed of base, sugar and phosphate group.

---

57) Which of the following terms (bacteriophage, thymine, guanine, nucleosome, nucleotide) matches the definition: pairs with cytosine.

---

58) Which of the following terms (bacteriophage, thymine, guanine, nucleosome, nucleotide) matches the definition: pairs with adenine.

---

59) Which of the following terms (bacteriophage, thymine, guanine, nucleosome, nucleotide) matches the definition: virus infecting bacterial cell.

---

60) Which of the following terms (bacteriophage, thymine, guanine, nucleosome, nucleotide) matches the definition: DNA wound round histone core.

---

61) Which of the following terms (replication, acylation, translation, translocation, transcription) matches the definition: movement of ribosome along mRNA.

---

62) Which of the following terms (replication, acylation, translation, translocation, transcription) matches the definition: synthesis of RNA from DNA.

---

63) Which of the following terms (replication, acylation, translation, translocation, transcription) matches the definition: synthesis of DNA form DNA.

---

64) Which of the following terms (replication, acylation, translation, translocation, transcription) matches the definition: attachment of amino acid to tRNA.

---

65) Which of the following terms (replication, acylation, translation, translocation, transcription) matches the definition: conversion of language of nucleotides to language of amino acids.

---

66) Which of the following molecules (polymerase, rRNA, mRNA, DNA, tRNA) matches the function: carries coded information for specifying polypeptide to be made.

---

67) Which of the following molecules (polymerase, rRNA, mRNA, DNA, tRNA) matches the function:carries amino acid to ribosome.

---

68) Which of the following molecules (polymerase, rRNA, mRNA, DNA, tRNA) matches the function:necessary for transcription to occur.

---

69) Which of the following molecules (polymerase, rRNA, mRNA, DNA, tRNA) matches the function: major component of ribosome.

---

70) Which of the following molecules (polymerase, rRNA, mRNA, DNA, tRNA) matches the function: carries information for protein complexed with histones.

DIRECTIONS: FOR ESSAYS, FOLLOW DIRECTIONS GIVEN BY YOUR INSTRUCTOR.

71) ESSAY --- Compare the three types of RNA regarding formation of the mature RNA, size, three dimensional structure, and role in protein synthesis. (5 points)

72) ESSAY --- In a certain strain of bacteria, the operation site of an operon containing the structural genes for enzymes X, Y, Z, has been deleted as a result of a mutation. Enzymes X, Y, and Z are inducible under specific conditions. What is the effect of this deletion of the operator site on the production of these enzymes? (5 points)

80-90 " _____ THEORY" by _____ & _____.

ANSWER KEY FOR: A.P. BIOLOGY CHAPTER 9&10 FORM: #201

INSTRUCTOR: _____ DATE: _____

CLASS: _____

If you are using alternate forms, be sure that the FORM # on this answer key matches the student's answer sheet form #.

1) - - C - -
2) - B - - -
3) - B - - -
4) A - - - -
5) - - C - -
6) A - - - -
7) - - C - -
8) - B - - -
9) - B - - -
10) - - C - -
11) - B - - -
12) - - - D -
13) - - - D -
14) - B - - -
15) - - - D -
16) - B - - -
17) - - C - -
18) - - C - -
19) - - C - -
20) - B - - -
21) - - - D -
22) A - - - -
23) - - - D -
24) - - - D -
25) - - - D -
26) - B - - -
27) - - C - -
28) - - C - -
29) A - - - -
30) A - - - -
31) - B - - -
32) - - C - -
33) A - - - -
34) - F
35) - F
36) - F

37) T -
38) - F
39) - F
40) T -
41) T -
42) - F
43) - F
44) - F
45) T -
46) - F
47) - F
48) - F
49) T -
50) - F

MATCHING (SHORT-ANSWER) QUESTIONS:

51) McClintock

52) Watson and Crick

53) Chargraff

54) Franklin

55) Griffith

56) nucleotide

57) guanine

58) thymine

59) bacteriophage

60) nucleosome

61) translocation

62) transcription

63) replication

64) acylation

65) translation

66) mRNA

67) tRNA

68) polymerase

69) rRNA

70) DNA

ESSAY QUESTIONS:

71) CHAPTER 10, QUESTION #2
72) CHAPTER 10 QUESTION #3

A.P. BIOLOGY CHAPTER 11 FORM: #301

INSTRUCTOR: _____ DATE: _____

CLASS: _____

DIRECTIONS: FOR EACH QUESTION, CIRCLE THE ONE BEST ANSWER.

1) First Letter of a restriction enzyme stands for the:

A. Strain of the organism
B. Genus of the organism
C. Specific epithet
D. None of these

2) Inserting a plasmid into a cell capable of generating many copies of itself, this is called:

A. Cloning B. Endonuclease C. Recombination D. All of these

3) All fragments of DNA that are separated by gel electrophoresis are influenced by all the following except:

A. Charge of the molecule
B. Strength of the electrical field
C. Density of the gel
D. PH of the buffer solution

4) Which substance was the inducer of the Lac Operon Gene:

A. LB AGAR B. JM101 STRAIN C. X-GAL D. IPTG

5) LB+ plates contain:

A. JM101 Strain B. pUC 18 plasmid
C. ampicillin D. none of these

6) The enzyme which joins two pieces of DNA together is called

A. restriction endonclease. B. ligase.
C. polymerase. D. reverse transcriptase.

7) To create complementary sticky ends, two pieces of DNA are treated with the same

A. restriction endoclease. B. ligase.
C. polymerase. D. reverse transcriptase.

8) Extrachromal pieces of DNA in bacterial cells are called

A. plasmids B. clones C. oncogenes. D. vectors.

9) Restriction endonuclease

A. are produced only by E. COLI.
B. protect the bacterium against bacteriophages.
C. cut only one strand on a piece of DNA.
D. join two pieces of DNA together.

10) Cancer cells are characterized by

A. immorality. B. lack of contact inhibition.
C. uncontrolled growth. D. all of the above.

11) Which of the following might be a step in carcinogenesis?

A. base substitution resulting from exposure to a mutagen
B. viral infection
C. chromosomal rearrangement
D. all of the above

12) In order to join human DNA and cow DNA, both must first be treated with the same

A. DNA polymerase. B. DNA ligase.
C. reverse transcriptase. D. restriction endonuclease.

13) The formation of hybrid nucleic acids depends on the

A. fact that DNA will anneal with DNA but not RNA.
B. association of identical strands of DNA.
C. weakness of the covalent bonds between the bases in DNA.
D. strands having a high degree of complementary.

14) It may be concluded from experiments demonstrating totipotency and from nuclear transplantation that

A. gene loss accompanies differentiation.
B. differentiation results from differential gene expression.
C. germ cells have the haploid number of chromosomes.
D. no cell is totipotent expect a malignant cell.

15) DNA can be introduced into the host cell by

A. viruses. B. plasmids. C.mutagens. D. a and b are correct.

16) Restriction endonuclease cleave

A. hydrogen bonds between bases.
B. covalent bonds between the sugar and phosphate.
C. ionic bonds between A and T, and C and G.
D. hydrogen bonds between deoxyribose and phosphate.

17) Consider the DNA sequence: A A T C G G C T A T. Which of the following sequences would form a hybrid with the given molecule?

A. A A T C G G C T A T B. T T A G C G G A T A
C. T A A G G C G A T A D. U U A G C C G A U A

18) In order for the two DNA molecules to form a recombinant DNA, they must

A. be from the same organism.
B. be the same length.
C. have complementary sticky ends.
D. all of the above.

19) Bacterial DNA is not cleaved by restriction endonuclease because it is

A. circular B. covered with histones
C. methylated. D. coiled very tightly.

20) EcoRI, a restriction enzyme, recognizes the sequence GAATTC.
CTTAAG
Into how many fragments will EcoRI cut the double helix of DNA presented below?

DNA HELIX: C C G A T T C C G C T C G G A A T T C G A T T
G G C T A A G G C G A G C C T T A A G C T A A

A. 0 fragments B. 1 fragment C. 2 fragments D. 3 fragments

21) Recombinant DNA technology is possible because of

A. specific base pairing rules.
B. the universality of the genetic code.
C. restriction endonuclease.
D. all of the above.

22) Which of the following limit the usefulness of recombinant DNA technology as a tool for obtaining human gene products from bacterial cells?

A. the lack of specificity of reverse transcriptase.
B. the lack of bacteria which take up vectors
C. the lack of the appropriate regulatory aequences and signals.
D. all of the above.

23) Nucleic acid hybridization

A. can occur only between two identical DNA strands.
B. requires breaking the sugar-phosphate backbone.
C. can be used to determine evolutionary relationships between different species and kingdoms.
D. cannot occur between DNA and RNA.

24) A pathologist analyzed a tumor surgically removed from a large intestine of a 60 year old man. She performed a series of tests to determine whether or not the tumor was malignant. Cells from the tumor exhibit the same pattern of growth in a culture medium as normal cells from the large intestine. Which of the following characteristics would you expect to be true for these cells?

A. difference in morphology, compared to normal cells
B. presence of chromosomal rearrangements or extra chromosomes
C. changes in the cell's motility
D. none of the above.

25) Restriction endonclease, EcoRI, recognizes the sequence GAATTC
CTTAAC
A recombinant DNA molecule can be made between this short piece of DNA and which of the following sequences?

A. C T A C C G G T A T
G A T G G C C A T A
B. G A A T T C A G C G
C T T A A G T C G C
C. C A A T T G A T C G
G T T A A C T A G C
D. A A C C G A A T T G
T T G G C T T A A C

DIRECTIONS: FOR EACH QUESTION, CIRCLE THE ONE BEST ANSWER.

26) TRUE or FALSE --- Hybridization occurs only between complementary DNA single strands and cannot occur between DNA and RNA.

27) TRUE or FALSE --- Only DNA from the same or similar organisms can be combined to form a hybrid molecule.

28) TRUE or FALSE --- Restriction enzymes randomly cut DNA molecules.

29) TRUE or FALSE --- An oncogene functions in normal growth and embryogenesis, but has the potential to cause cancer.

30) TRUE or FALSE --- Cellular transformation to a malignant state results from changes in the cell's DNA.

31) TRUE or FALSE --- The sugar-phosphate backbone is destroyed by heating DNA to 100 degrees Centigrade.

32) TRUE or FALSE --- Metastasis is a characteristic of malignant cells.

33) TRUE or FALSE --- A piece of DNA 10,00 nucleotides long moves farther when subjected to electrophoresis than a piece of DNA 5,00 nucleotides long.

34) TRUE or FALSE --- Any human gene transplanted or transferred into a bacterial cell will be expressed.

35) TRUE or FALSE --- Some retroviruses contain oncogenes of animal origin.

DIRECTIONS: FOR EACH QUESTION, WRITE YOUR ANSWER ON THE LINE PROVIDED.

36) Which of the following terms (vector zygote, oncogene, clone, plasmid) matches the definition: cancer causing gene.

---

37) Which of the following terms (vector zygote, oncogene, clone, plasmid) matches the definition: group of genetically identical organisms or cells.

---

38) Which of the following terms (vector zygote, oncogene, clone, plasmid) matches the definition: fertilized egg.

---

39) Which of the following terms (vector zygote, oncogene, clone, plasmid) matches the definition: small circular DNA molecule in bacterial cell.

---

40) Which of the following terms (vector zygote, oncogene, clone, plasmid) matches the definition: carrier of DNA.

---

41) Which of the following enzymes (DNA polymerase, reverse transcriptase, restriction enzyme, RNA polymerase, DNA ligase) matches the function: cutting enzyme.

---

42) Which of the following enzymes (DNA polymerase, reverse transcriptase, restriction enzyme, RNA polymerase, DNA ligase) matches the function: joining enzyme.

---

43) Which of the following enzymes (DNA polymerase, reverse transcriptase, restriction enzyme, RNA polymerase, DNA ligase) matches the function: makes DNA from RNA.

---

44) Which of the following enzymes (DNA polymerase, reverse transcriptase, restriction enzyme, RNA polymerase, DNA ligase) matches the function: makes DNA from RNA.

---

45) Which of the following enzymes (DNA polymerase, reverse transcriptase, restriction enzyme, RNA polymerase, DNA ligase) matches the function: makes RNA from DNA.

DIRECTIONS: FOR ESSAYS, FOLLOW DIRECTIONS GIVEN BY YOUR INSTRUCTOR.

46) ESSAY --- How do you think it was determined that retroviruses carry genetic material of cellular origin?

47) ESSAY --- Consider the following situation. You have ten clones of bacteria each of which has a different piece of human DNA inserted into a plasmid. From these ten clones you want to select the one which has the gene for protein A. In each of the clones, the DNA in the plasmid is isolated and made into a single stranded molecule. This DNA is exposed to radioactively labeled mRNA for protein A. The following results were obtained:

clone #	1	2	3	4	5	6	7	8	9	10
reaction with mRNA	+	-	-	-	+	-	-	-	+	-

(+) indicates radioactivity associated with DNA
(-) indicates no radioactivity associated with DNA

a. Which clone(s) contain the gene for protein A?
b. Why can mRNA be used as a "probe" for locating the gene for protein A?

ANSWER KEY FOR: A.P. BIOLOGY CHAPTER 11 FORM: #301

INSTRUCTOR: _____ DATE:_____

CLASS: _____

If you are using alternate forms, be sure that the FORM # on this answer key matches the student's answer sheet form #.

1) - B - - -
2) A - - - -
3) - - - D -
4) - - - D -
5) - - C - -
6) - B - - -
7) A - - - -
8) A - - - -
9) - B - - -
10) - - - D -
11) - - - D -
12) - - - D -
13) - - - D -
14) - B - - -
15) - - - D -
16) - B - - -
17) - - - D -
18) - - C - -
19) - - C - -
20) - B - - -
21) - - - D -
22) - - C - -
23) - - C - -
24) - - - D -
25) - B - - -
26) - F
27) - F
28) - F
29) T -
30) T -
31) - F
32) T -
33) - F
34) - F
35) T -

MATCHING (SHORT-ANSWER) QUESTIONS:

36) oncogenes

37) clone

38) zygote

39) plasmid

40) vector

41) restriction enzyme

42) DNA ligase

43) reverse transcriptase

44) DNA polymerase

45) RNA polymerase

ESSAY QUESTIONS:

46) CHAPTER 11, QUESTION #1
47) CHAPTER 11, QUESTION #2

APPENDIX H

"DRY LAB" ON PLASMIDS

PLASMID LAB

OBJECTIVE: To observe the process of recombinant DNA technology and visualize the product on paper.

BACKGROUND INFORMATION:

Biotechnology includes three technologies that all use living organisms to carry out chemical processes or to produce substances. Current biotechnology includes bioprocesses, monoclonal antibodies, and recombinant DNA technology. Bioprocesses are part of the manufacture of bread, cheese, and beer. They may also be used to break-down sewage wastes.

Monoclonal antibody technology allows the fusion of cancer cells that reproduce very frequently with cells that produce a particular antibody. The new cells, hybridomas, are cloned to produce the antibody in quantity.

Recombinant DNA technology also produces some substance in large quantities. A gene coding for a particular protein is transferred into a host bacteria. This bacterium multiplies and produces the protein in volume.

MATERIALS FOR THE LAB: scissors and glue

STEPS FOR RECOMBINATION:

A. The scientists must identify the gene that codes for the production of the protein they want to manufacture. One way is to work backwards from the amino acid sequence of the desired protein to the nucleotide sequence of the gene. The gene must be isolated, that is removed from the host chromosome. *Restriction enzymes, or endonucleases*, from bacterial cells are used. Bacteria normally use these to destroy foreign DNA that gets into the bacterial cell. These enzymes recognize and cut specific sequences of the DNA. The cut is a staggered one, called a *sticky end*. These ends will automatically bind with complementary base pair sequences on other DNA strands cut with the same enzyme. The enzyme can be used to cut on either side of the gene desired.

B. Now begins the process of trying to get the gene into a host bacteria cell. A small circular piece of DNA that is a normal component of bacteria is used to carry the gene. Plasmids have a region, called the replication origin, that enables them to be replicated. After removing a plasmid from a bacterial cell, scientists cleave the plasmid using the same enzyme they used to clip out the gene. This way the sticky ends of the plasmid will match those of the gene. It is important to use restriction enzymes that do not cut within the gene itself. The cleaved plasmid and the cleaved gene are mixed together. The sticky ends of the gene and the sticky ends of the plasmid come together and their complementary bases pair with hydrogen bonds. A DNA ligase enzyme is added, which completes the bond.

C. The new plasmid is mixed with the host bacteria, and is taken up by the bacteria. This process is called transformation. To check that the plasmid has been taken up, they test the bacteria for some basic characteristic of the plasmid. For example, if a plasmid produces resistance to an antibiotic, scientists could spread the bacteria they hope contain the

plasmids on a petri dish of agar mixed with antibiotic. Only the bacteria containing plasmids with antibiotic resistance and the replication origin will survive. Then it would be tested for the presence of the gene. The plasmids are replicated within the host and begin producing the protein.

LAB PROCEDURE:

1. CONSTRUCT THE PLASMID. The strips on the plasmid sheet are written with the DNA 3' to 5' from top to bottom on the left-hand side of the strip and from 5' to 3' from top to bottom on the right-hand side of the strip. Some of the base pairs have been left out. Fill in the correct matching base pairs first. Cut out the plasmid strips along the dotted lines and put them together end to end in any order. You may even discard one of the strips if you choose. Be sure your plasmid still contains the replication origin. Tape your final plasmid into a circle shape.

2. LOCATE RESTRICTION SITES. Use your enzyme sheet to compare the sequences of base pairs on the cards with what you have on your plasmid. Mark a starting point on the plasmid and work your way around, writing the restriction sites on your plasmid as you find them. Draw a diagram, or restriction map, of the plasmid. Mark the antibiotic sites, restriction sites, and the replication origin in relative distance to one another on a circle.

3. ASSEMBLE YOUR DNA AND REMOVE THE GENE. Cut out the strips on the cell DNA sheet and assemble the strips in the order one through six, as indicated on the handout, to end up with a long flat strip. The gene starts with the code for methionine and ends with a stop codon. Determine which restriction sites occur above and below the gene. Match these to the ones on the plasmid. Use one enzyme to cut the DNA above the gene and then cut your plasmid with the same enzyme. Find a second enzyme to cut below the gene and use this enzyme to cut the plasmid again. Be sure the portion of the plasmid that remains contains the origin and at least one antibiotic resistance site.

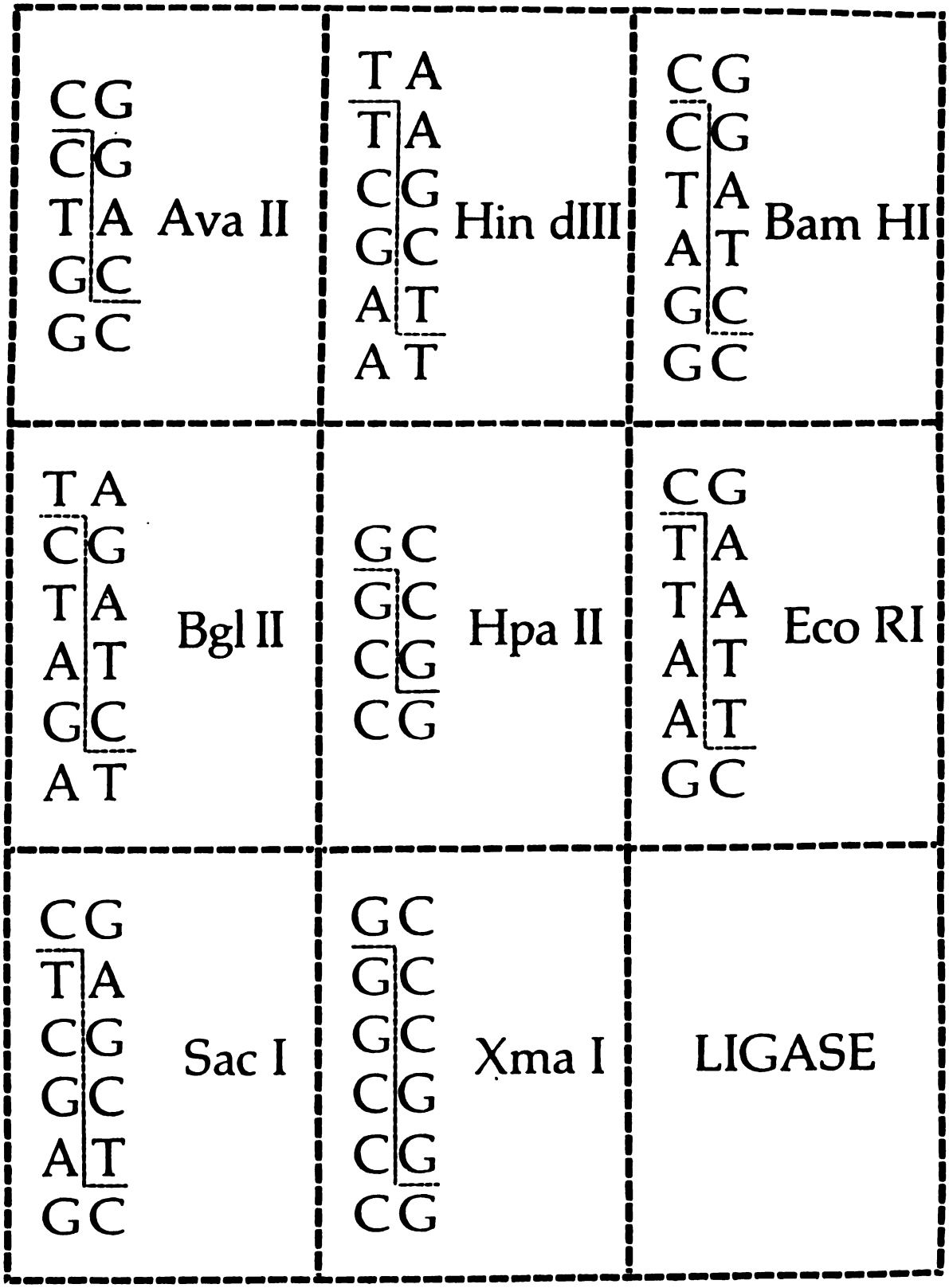
4. ASSEMBLE FINAL PLASMID. Mix the gene fragment with the plasmid fragment and use the DNA ligase to bond the sticky ends making a complete plasmid containing the gene.

5. DETECTING THE PLASMID. In a lab situation, the recombinant DNA plasmids are mixed with the host bacterial cells and could be followed by screening for the antibiotic resistance and the protein produced. Decide which antibiotic or antibiotics you will use to detect your plasmid.

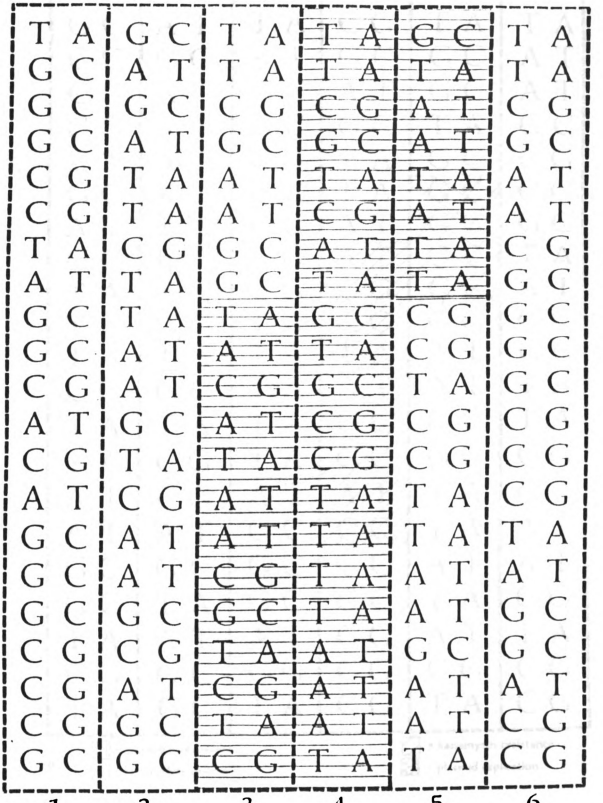
6. QUESTIONS:

a. Which enzymes did you use?
b. Which antibiotics would you use to test for the presence of the plasmid?
c. What is a gene?
d. Why is the plasmid a circle while the DNA was not?
e. What are the three types of biotechnology?
f. Why do you think bacteria naturally contain plasmids?
g. What are hybridomas and how do they help modern medicine?
h. How can recombinant DNA technology be a benefit? a threat?

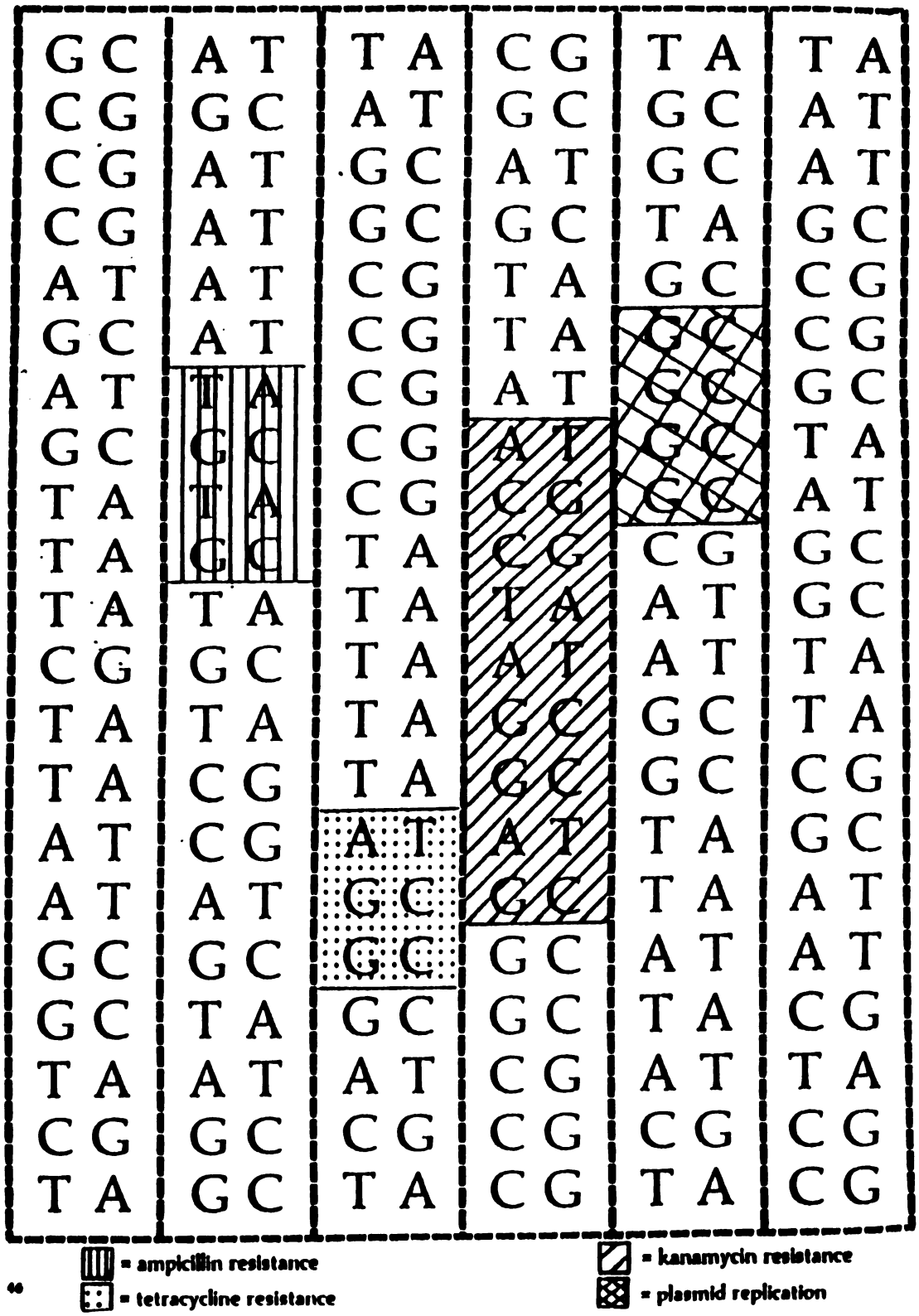
Enzymes


C G
C G
T A
G C
G C
Ava II
T A
T A
C G
G C
A T
A T
Hin dIII
C G
C G
T A
A T
G C
G C
Bam HI
T A
C G
T A
A T
G C
A T
Bgl II
G C
G C
C G
C G
Hpa II
C G
T A
T A
A T
A T
G C
Eco RI
C G
T A
C G
G C
A T
G C
Sac I
G C
G C
G C
C G
C G
C G
Xma I
LIGASE

Cell DNA



Plasmid


= ampicillin resistance
= tetracycline resistance
= kanamycin resistance
= plasmid replication

Part A:

1. What was the goal of the procedure?
2. What was represented by the first chain of beads?
3. Why were the four beads removed? What did they represent?
4. What removes these in nature?
5. What in nature forms the DNA from the mRNA?
6. From what was this molecule first obtained?

Part B:

7. You constructed a plasmid. From what do plasmids come?
8. What cuts the plasmid?
9. From what does this molecule come?
10. Why must the same cutting molecule be used on both the plasmid and the DNA?
11. Why was HindIII used?
12. What is HindIII?
13. Why was the loss of the four beads from each end of the DNA no loss to the coded message it carries?
14. What two steps would follow these?

APPENDIX I

COMPARISON OF PRETEST & POST TEST SCORES

VRSN-10/15/9017.5 MSU COMPUTER LAB GRADER III RUN DATE 91/07/24 2

RAW SCORE DISTRIBUTION

)B NBR-2301 FILE-9231 COURSE-NSC850 TEST NO-01 FORM- A

RAW SCORE	FREQUENCY	CUMMULATIVE FREQUENCY	PERCENTILE RANK	STANDARD SCORE
29	1	1	95	66.9
27	1	2	87	63.5
23	2	4	75	56.5
22	1	5	62	54.7
20	1	6	54	51.3
19	1	7	45	49.5
16	1	8	37	44.3
14	2	10	25	40.8
12	2	12	8	37.3

TOTAL ITEMS ON TEST 45 MEAN 19.25 STANDARD DEVIATION 5.74 VARIANCE 32.93

STANDARD SCORE HAS MEAN OF 50 AND STANDARD DEVIATION OF 10

VRSN-10/15/9017.5 MSU COMPUTER LAB GRADER III RUN DATE 91/07/24 2

RAW SCORE DISTRIBUTION

B NBR-2302 FILE-9232 COURSE-NSC850 TEST NO-01 FORM- B

RAW SCORE	FREQUENCY	CUMMULATIVE FREQUENCY	PERCENTILE RANK	STANDARD SCORE
35	1	1	95	66.1
34	1	2	86	63.0
31	2	4	72	53.4
30	2	6	54	50.2
29	3	9	31	47.1
28	1	10	13	43.9
23	1	11	4	28.0

TOTAL ITEMS ON TEST 45 MEAN 29.91 STANDARD DEVIATION 3.15 VARIANCE 9.89

STANDARD SCORE HAS MEAN OF 50 AND STANDARD DEVIATION OF 10

BIBLIOGRAPHY

BIBLIOGRAPHY

Anderson, John N.,1987, "A Basic Laboratory Course in Modern Biology", Modern Biology Series, Purdue University.

Arms, Karen and Camp,Pamela, 1990, Biology: Journey of Life(1st Edition), W.B. Saunders Company, 1990.

Baltimore, David,1984, The Brain of a Cell, Science 84 5(A): 149-151.

Bishop, Michael J.,1987, The Molecular Genetics of Cancer, Science: 235: 305-311.

Bloom, Mark,1990, Mapping and Sequence the Human Geome, Cabisco Biotechnology Tips, Volume 4 #1.

Campbell, Neil,1987, Biology, Benjamin/Cummings Publishing Co.

Campbell, Neil,1990, Biology, Benjamin/Cummings Publishing Co.

College Board, Advance Placement Course Description Booklet - Biology, 1989,1990, and 1991.

Cook, James L.,1987, Plasmid Lab, The Science Teacher 37:34-38.

Dixon, Linda,1988, teaching Recombinant DNA Technology in High School Biology Course, American Biology Teacher: 50: 368-373.

Glover, D. M., 1980, Genetic Engineering - Cloning DNA, Chapman and Hall Publishers.

Guilfoile, Patrick,1989, A simple DNA Isolation Technique using Halophilic Bacteria, American Biology Teacher, 51(3): 170-171.

Hagerman, Howard, national Science Foundation Workshop Summer 1991, Kellogg biological Station, michigan State University, July 1991.

Helms, Doris and Coker, Phillip,1989,Laboratory Manual for Students, Worth Publisher's

Kornberg, Arthur,1974, DNA Synthesis, W.H. Freeman and Co.

Lewis, Ralph W.,1988, Biology: A Hypothetic-Deductive Science, American Biology Teacher, 50(6): 362-366.

Micklis, David and Freyer, Greg, 1989, A Laboratory Introduction to DNA Restriction Analysis, Biotechnology Education: 1(1): 16-22.

Micklins, David and Freyer, Greg,1990,DNA Science: A first Course in Recombinant DNA Technology, Cold Spring Harbor Laboratory Press.

Myers, Richard,1988, Recombinant DNA Technology in High School Biology Laboratory, American Biology Teacher: 50(1): 43-45.

Ogden, Richard and Adams, Deborah,1989, Recombinant DNA Technology: Applications, Carolina Tips, May 1st.

Old, R.W. and Primrose, S.B., 1981, Principles of Gene manipulation, University of California Press.

Sambrook, J., Fritsch E. F., Maiatis, C.,1989, Molecular Cloning: A Laboratory Manula, 2nd Edition. Cold Spring Harbor Lab Press.

Sharp, Charles,1989, Use of Methylene Blue for Staining DNA, National Science Workshop:Molecular Biology, Michigan State University.

Smith, Richard G., 1990, Recombinant DNA Workstation, Lofstrand Labs Limited.

Stone, Harry J.,1982, Biotechnology, Carolina Tips, 45 (11).

Stryer, Lubert, 1986, Biotechemistry, 3rd edition.

Suelter, Clarence, 1989, National Science Foundation Workshop:Molecular Biology, Michigan State University.

Thompson, Robert G.,1988 Recombinant DNA Made Easy: Jumping Genes, American Biology Teacher, 50(2): 101-106.

Thompson, Robert G., 1989, Recombinant DNA Made Easy II: Gene, Gene, Who's Got the Gene?, American Biology Teacher, 51(4): 226-232.

Vigue, C.,1984, Oswald, Avery, and DNA, American Biology Teacher, 46: 207-211.

Watson, James D.,1989,For a Few Dollars More, Biotechnology Education: 1(1): 3-5.