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# EXPLORING GENETICS AND DEVELOPMENTAL BIOLOGY USING MULTIDEIMENSIONAL MANIPULATIVES AND BIOTECHNOLOGY LABORATORIES

By

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## ABSTRACT

EXPLORING GENETICS AND DEVELOPMENTAL BIOLOGY USING MULTIDIMENSIONAL MANIPULATIVES AND BIOTECHNOLOGY LABORATORIES

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This study examines the effectiveness of biotechnology laboratories and manipulative models in increasing student understanding of genetics. The major goals for this unit included improving students' personal experiences by incorporating models to translate abstract biological concepts in the text into concrete learning and conducting biotechnology laboratories at research facilities, including the use of their own DNA. Data sources for evaluating the effectiveness of the unit included responses to questionnaires, written essays, pre and posttests performance in laboratory techniques, and field observations. The practice of using these student centered modeling activities and laboratory experiments produced significant gains relative to the use of critical thinking skills, laboratory techniques, and general attitude in learning genetic related topics.

## ACKNOWLEDGMENTS

I dedicate this thesis to all my Advance Placement biology students, past and future. I thank my former students for providing me with insight into how to be a better teacher, and I hope that these laboratories and activities will inspire future students with an appreciation of this exciting science. I wish to express my deepest appreciation to my husband, Chris, for his constant encouragement and patience. To Dr. Merle Heidemann, Ken Nadler, Roger Herr, and Dan O'Mally I wish to express my gratitude for their generous time and advice, without which, this thesis would not be possible. Finally, I'd like to thank David Thorne for his comprehensive and thoughtful editing.

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# INTRODUCTION

## STATEMENT OF PROBLEM AND RATIONALE

Advanced Placement Biology is a relatively new course at Grand Blanc High School. During the 1999-2000 school year I piloted a semester of the Advanced Placement College Board curriculum and found the lecture driven unit on genetics to be very difficult for the students, especially the biotechnology section. Student's grapple with the concepts of molecular genetics because the details are abstract and most students have very little background knowledge of the subject. In addition, I was constrained by time the first year and did not teach developmental biology, which is part of the Advanced Placement curriculum. Therefore, my goal of this thesis was to research some effective teaching techniques and develop molecular genetics and developmental biology laboratories and activities. The tools and methods used to meet the objective included pretests; class discussions; lab drawings, labels, interpretation and assessment questions; an essay; and post-tests. In general, I chose to study the teaching of these topics because they are socially popular, conceptually difficult, vastly growing, and consistently asked on the AP exam.

Students can find aspects of genetics in the newspaper or magazines almost daily. Not only engaging because of importance in modern culture, genetics has the power to inspire. What once seemed only to be science fiction is coming within grasp as we move closer to understanding how the human genome is sequenced, genetic diseases are caused, animals are cloned, and organisms are genetically modified. New technology, clever choices of model organisms, and collaboration have made molecular genetics a

necessary tool for researching almost any field related to biology, including developmental biology.

These really are the best and most challenging times for teaching and learning about the "new" biology. The challenge emerges as a paradox because the geyser of information that makes molecular genetics so exhilarating also threatens to drown students under the volume of details. In addition, the details are abstract, which makes teaching them even more difficult. It seems like the biology texts keep getting bigger, but the academic year remains relatively constant in comparison. "So much biology, so little time!" (Schofield, 2000)

Moreover, the study of genetics and animal development is important for students taking the Advanced Placement exam. These two topics comprise over ten percent of the AP Program's course outline. Considering the fact that questions on the Advanced Placement Biology exam are generated from the Advanced Placement College Board course outline, it is more than advantageous to teach them well!

## LITERATURE REVIEW OF PEDAGOGY

I realized during my first year teaching AP biology that my students found the abstract details of the molecular genetics and developmental biology section very difficult. During the summer of 2000, through a course of study with the Division of Math and Science Education within the College of Natural Science at Michigan State University, I researched and implemented several teaching methods and to encourage students to experience science by active participation. In other words, to give students the opportunity to "do" quantitative and qualitative science, ensuring that they get the "big picture" and major insights into the science of life.

**Piaget:**

The first instructional theory incorporated into the teaching unit was by Piaget. Piaget and others have demonstrated that the sequence of topics and activities and their degree of familiarity to the students must be taken into consideration in constructing curriculum (Thompson, 1998). Providing activities or situations that challenge and engage learners and require adaptation to new materials facilitates cognitive development. This adaptation includes both assimilation and accommodation. Assimilation involves the interpretation of events in terms of existing cognitive structure, whereas accommodation refers to changing the cognitive structure to make sense of the environment (Kearsley, 2001).

**Learning Cycle:**

In the Learning Cycle, students become familiar with new topics and phenomenon through some concrete experience or lab activity (Thompson, 1998). These "hands-on" explorations produce the familiarity needed for students to further construct or understand important abstractions. Research has documented the problems traditional college freshmen have understanding abstract concepts (Gottfried et al. 1993). Moreover, studies have revealed that some students are still concrete thinkers and the majority of college sophomores still function in transitional stages that have not attained the abstract thinking level (Malacinski & Zell, 1996). When students have the opportunity to actively construct their own understanding through experimentation and discussion of information, rather than rote memorization of information, they can construct meaningful understanding. The process is more interesting to them and produces increased retention and comprehension.

**Authentic Laboratories:**

Given this explosion of biotechnology information, effective teaching methods are needed for advanced students. Students who conduct biotechnology laboratories have opportunities to acquire the techniques that scientists use when they study molecular biology and to improve their knowledge genetics and microbiology. Authentic laboratories are defined as exercises that utilize materials, equipment, and technology that are used in research, industrial and medical laboratories (Gillen and Chiappetta, 1996). Authentic laboratories are a powerful strategy for instruction because students are captivated by this technology and are eager to learn real world applications. Students often ask, "What good will this information do me? Why do I have to learn this?" They

prefer authentic laboratories to dry laboratories, which might include simulations, cut-and-paste paper activities, and computer exercises because they usually see the connection between school science and skills they may use in a technical career (Gillen and Chiappetta, 1996).

**Processing Framework:**

Lastly, The pedagogical literature research on strategies and methodologies for teaching science shows clear evidence that students in process-approach programs learn more than do students in traditional textbook-based programs (Ostlund and Thompson, 1998). Craik and Lockhart presented an interesting processing framework in 1972. According to their theory, stimulus information is processed at multiple levels simultaneously depending upon its characteristics (Kearsley, 2001). Teaching techniques that involve strong visual images or many associations with existing knowledge will be processed at a deeper level. Each new activity incorporates several enticing visual models or living organisms and the sophisticated laboratories require greater processing skills. Hopefully, as students internalize the experiences and process the information on genetics and development, they will remember it. In this time of ever-expanding biology, teachers have no choice but to make their courses less encyclopedic in content and more experiential in process: "less is more" (Schofield, 2000).

## DEMOGRAPHICS OF CLASSROOM

The unit discussed in this thesis for teaching genetics and developmental biology to Advanced Placement students was implemented at Grand Blanc High School, a member of the Genesee County Intermediate School District in Michigan. AP biology is intended to be equivalent to an introductory biology course found at the freshman university level. It is a two-semester course and reflects the outline provided by the College Board emphasizing molecules and cells, heredity, molecular genetics, evolutionary biology, diversity of organisms, structure and function of plants, and ecology. There was only one class of Advanced Placement biology and this was the second year in a row it has been taught at Grand Blanc by Ron Calo, a biology teacher and myself. We divided the course contents into two semesters.

Grand Blanc is in southeast Genesee County just a few miles south of Flint, Michigan. The city and township have a combined population of about 35,000 people. The individuals enrolled in this class were all senior students, with grade point averages above 3.5, who received an A in biology and chemistry, and were recommended by their biology teacher. Out of the 20 students in the class, there were 8 males and 12 females. The students in the class also had diverse ethnic backgrounds. Most of the students were motivated to enroll into AP biology because they are expecting to pursue careers in nursing, dental, medicine, engineering, and environmental science. Moreover, many of the students were also enrolled in several other Advanced Placement classes at the high school and a few were even taking college classes at the University of Michigan in Flint in the evenings.

## HISTORICAL PERSPECTIVES OF TOPICS TAUGHT

One of the best books I have read as a teacher is The Science Class You Wish You Had, by David and Arnold Brody. It clearly documents the seven greatest scientific discoveries in history and the people who made them. Another great book I used for references below was the Random House Webster's Dictionary of Scientists. I have chosen these two books and the American Biology Teacher journal article "Biotechnology Outlines for Classroom Use" by Mary Jane Paolella as primary resources for the chronology, discoveries, and quotes that led to our present understanding of molecular biology, biotechnology and development.

Like so much else, the systematic study of living things began with the Greeks. Aristotle (384-323 BC) wrote several biological works, which laid the foundations for comparative anatomy, taxonomy, and embryology. A few millennia later, around 1672, Marcelle Malphigi undertook the first microscopic studies in embryology by describing the development of a chicken egg. Five years later Anton van Leeuwenhoek greatly improved the microscope and used it to describe spermatozoa as well as many microorganisms. In 1830, Johannes Muller discovered proteins. Three years latter Anselme Payen and JF Persoz first isolated an enzyme. In his 1858 book Cellular Pathology, Rudolf Virchow explained that "cells are the link in the great chain...formations that form tissues, organs, systems and the individual". In 1869, Friedrich Mieschler discovered DNA, although he did not know it was the genetic material. August Weismann wrote that the sperm and egg cells of animals must contain "something essential for the species, something which must be carefully preserved and

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passed on from one generation to another." This germ plasm theory was published in 1886.

Later in the 1900's, a Russian-born chemist, Phoebus Levene identified ribose as the sugar in RNA, and he identified certain components of DNA also. Starting in 1928, Frederick Griffith discovered that a certain unknown substance from the cells of one strain of dead pneumococci was able to enter a different and living strain and cause the live strain to pass the dead strain's hereditary characteristics to the live strain's offspring. Avery, MacLeod, and McCarty first demonstrated the transforming role of DNA in genetic inheritance in 1943. In 1950 Erwin Chargaff determined the proportionate amounts of the deoxynucleotide bases in each molecule of DNA: guanine equals cytosine and adenine equals thymine. Genetic engineering consists of a collection of methods used to manipulate and transfer genes. The first of these techniques dates back to 1952 when Joshua Lederberg found that bacteria exchange genetic material contained in a body he called a plasmid. In 1953 Francis Crick and James Watson suggested that deoxyribonucleic acid was the hereditary material based on its structure. That same year William Hayes established that plasmids were rings of DNA free from the main DNA in the chromosome of the bacteria. George Beadle, Edward Tatum and Joshua Leaderberg shared a Nobel price in 1958 for the formation of "one gene one enzyme hypothesis", which means each gene dictates the production of one enzyme. Beadle also showed how a bacteriophage could bring about the transfer of DNA by transduction. Working at the Matthew Meselson and Franklin Stahl carried out their successful experiment with *E. coli* to prove the semiconservative nature of DNA replication in 1957. In the semiconservative model the two strands of the parental molecule separate, and each

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functions as a template for synthesis of a new complementary strand. Watson and Crick first suggested the concept. In 1959, Arthur Kornberg won the Nobel Prize for his discovery of the enzyme DNA polymerase, which enabled molecules of the genetic material DNA to be synthesized.

In the next decade, 1962, Maurice Wilkens shared the Nobel Prize with Francis Crick and James Watson for his work on the molecular structure of nucleic acids, particularly DNA using X-ray diffraction. In 1965, researchers realized that genes conveying antibiotic resistance in bacteria are often carried on plasmids and two years later DNA ligase, which joins DNA segments together, was isolated. In 1969, Reiji Okazaki, suggested that one strand of DNA is synthesized as small pieces that are later joined together. Werner Arber, who studied viruses which infect bacteria (called bacteriophages), took the next step. He found that bacteria resist phages by splitting the phage DNA using enzymes. By 1968 Arber had discovered the enzymes produced by bacteria that split DNA at specific locations. In addition, he found that different genes that have been split at the same location by one of the restriction enzymes, as they are called, would recombine under certain conditions when placed together in the absence of the enzyme. The resulting product is called recombinant DNA.

Two major events related to molecular genetics occurred in 1970: Hamilton Smith discovered restriction endonucleases in bacteria that degrade phage DNA without harming its own cellular DNA; and Daniel Nathans produced the first restriction map by using restriction enzymes to cut DNA. In 1972, Paul Berg used the restriction enzyme EcoRI to produce the first recombinant molecule. Berg spliced and combined into a single hybrid the DNA from an animal tumor virus (SV40) and the DNA from a bacterial

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virus. The following year, Stanley H Cohen and Herbert W Brown transplanted a functioning gene between organisms and produced a hybrid with dual antibiotic resistance using restriction enzymes and ligases. They cut a chunk out of a plasmid found in the bacterium *Eschericia coli* and inserted it in the gap a gene created from a different bacterium.

The polymerase chain reaction, which allows scientists to amplify a specific DNA sequence, was invented by Dr. Kary Mullis in 1983. In addition, the sequence of lambda phage DNA was published that same year. In 1985, Alec Jeffreys designed genetic fingerprinting. The Human Genome Organization was established in 1988 in Washington, DC, with the aim of mapping the complete sequence of human DNA. 1988 Telomere (chromosome end) sequence having implications for aging and cancer research is identified.

In 1995, Craig Venter [The Institute for Genomic Research (TIGR)] and Hamilton Smith (Johns Hopkins University) announced the complete sequencing of two bacterial genomes. The 1.9-Mb *Haemophilus influenzae* genome was finished and all gaps closed in less than a year; funding from the DOE microbial genome project, administered by Jay Grimes, allowed the 580-kb *Mycoplasma genitalium* genome to be completed in 3 months by Claire Fraser's team at TIGR. In September 1997, a team of scientists led by Frederick Blattner (University of Wisconsin, Madison) reported completing the sequence of the 4.6-Mb *Escherichia coli* K-12 genome. Obtaining the complete DNA sequence of the E. coli genome has been a goal of the Human Genome Project, both to help develop sequencing and gene-finding technology and to facilitate studies on gene function and organization. More than 4200 E. coli genes have been identified, although

the functions of over one-third of them remain unknown. Finally, on Febuary 12, 2001 a working draft of the human genome sequence was published in major science journals. Information included initial analysis of the descriptions of the sequence generated by the publicly sponsored Human Genome Project and reported by the private company, Celera Genomics.

## LITERATURE REVIEW OF SCIENTIFIC BACKGROUND

The following background information is needed by students to fully understand and appreciate the genetics and development unit. I present this information in the form of PowerPoint lecture notes, overhead transparencies, and prelaboratories, which are computer simulations. This information is intended to enhance the information in student textbooks. The laboratories and activities designed in this unit can easily be inserted into the Advanced Placement College Board curriculum, which includes information on DNA structure, replication, assimilation, separation, amplification and vertebrate development (Table 1).

**DNA Structure:**

A single strand of DNA consists of a backbone made of phosphate groups alternating with the pentose sugar deoxyribose. Linked to the 1'carbon of each sugar is one of four nitrogenous bases. The purine bases, adenine and guanine, have two ring structures whereas the pyrimidine bases, thymine and cytosine, have only one ring structures. The 5' phosphate of a deoxyribose is attached to the 3'OH of the adjacent deoxyribose to form a phosphodiester linkage (Watson and Crick, 1953). Moreover the 5' and 3' ends indicate polarity.

**DNA Replication:**

Before replication, the parent molecule has two complementary strands of DNA. Each base is paired by hydrogen bonding with its specific partner, adenine (A) with

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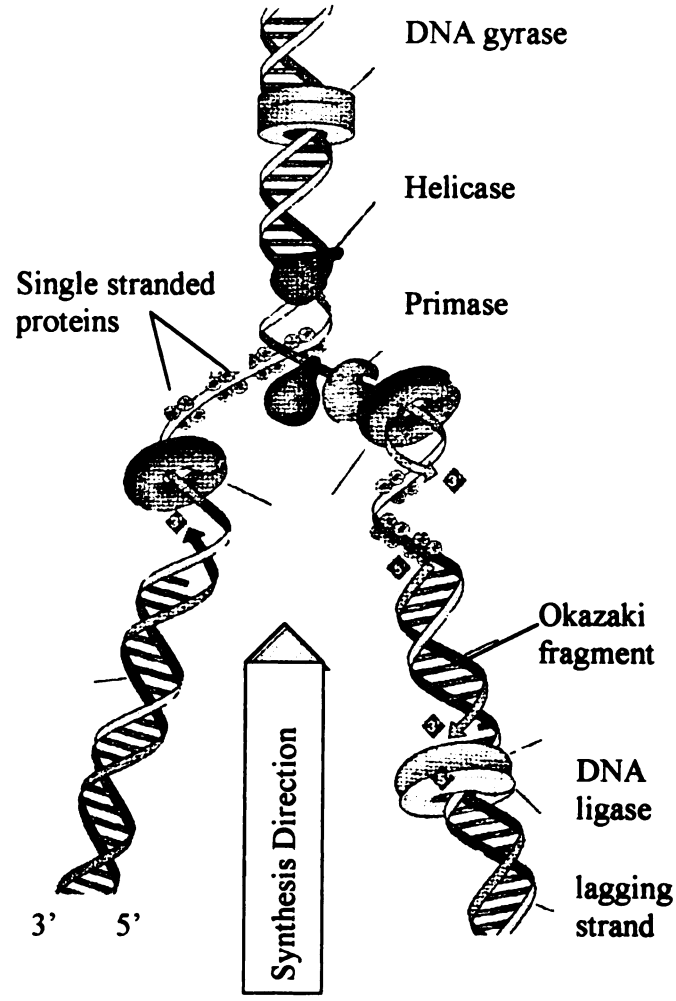
thymine (T) and guanine (G) with cytosine (C). The first step in replication is separation of the two DNA strands. Each original strand now serves as a template that determines the order of nucleotides along new complementary strands. Nucleotides bond at specific sites along the template surface according to the base-pairing rules. The nucleotides are connected to form the sugar-phosphate backbones of the new strands. Each DNA molecule now consists of one old strand and one new strand. This is called the semi-conservative model of DNA replication because the two strands of the parental molecule separate, and each functions as a template for synthesis of a new complementary strand. The result is two DNA molecules identical to the parent molecule. (Campbell 2000).

Figure 1: DNA Replication model

DNA synthesis begins at a specific base sequence termed the origin of replication. The double strands are separated at the origin of replication by DNA helicase and unwound by DNA gyrase revealing single-stranded regions (Atherly, 1999). These regions are immediately bound by single-stranded binding proteins, which prevent the single stranded regions from reforming into double strands. This area of active DNA synthesis is referred to as the



"replication fork" (Campbell, 2000). It is formed at the junction of the single strands and the double-stranded region. New strands are synthesized in a 5'→ 3'direction near the vicinity of the fork (Campbell, 2000). The leading strand is synthesized continuously in the direction toward the replication fork, whereas the lagging strand is synthesized discontinuously in a direction away from the replication fork. Both the leading and lagging strands require an RNA primer provided by the primase because DNA polymerase elongation can only proceed by addition to a free 3'OH end of an existing polynucleotide strand and cannot initiate a new strand (Solomon, 2000). The new complementary strands are synthesized by polymerase III. On the lagging strand, multiple initiations must occur because of the 5'→ 3' synthesis requirements. As a consequence, short segments of DNA are synthesized, which are called Okasaki fragments. To join these fragments the RNA primer is degraded to free nucleotides and another DNA polymerase fills in the gaps with deooxynucleotides. DNA ligase then catalyzes formation of the phosphoester bond between the 5'phosphate and 3'hydroxyl to yield a continuous strand of complementary DNA (Atherly, 1999).

**DNA Assimilation/ Bacterial Transformation:**

Bacterial plasmids are a separate, smaller (than the main bacterial DNA), circular DNA molecule that can be isolated and introduced into other cells by transformation. Transformation involves incorporation of genetic material into a cell, thereby changing its phenotype. The bacterial plasmid pAMP is used as a vector to transfer antibiotic resistance genes into E. coli (Advanced Placement Biology Development Committee, 1997). Restriction endonucleases are used to insert the gene into the plasmid. Restriction

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enzymes cleave the DNA at specific sequences of nucleotides, producing cuts that are usually jagged, with one strand of the DNA molecule extending beyond the second strand (Solomon, 1999). If both the plasmid and the foreign DNA, with the gene of interest, are treated with the same restriction enzyme, the jagged ends (sticky ends) of the foreign DNA will match the sticky ends of the plasmid DNA. DNA ligase can then bond the foreign DNA fragment to the plasmid DNA.

The transformation procedure begins by treating E. coli with $Ca^{2+}$, which makes them competent to take up DNA (Rapoza, 1999). Once the E.coli is competent, then a heat-shock is given to facilitate the absorption of DNA (Advanced Placement Biology Development Committee, 1997). Lastly, transformation is tested by treating the bacteria with ampicillin because only the E.coli cells that absorbed the pAMP will survive (Helms, 1994). This is because the plasmid, pAMP, carries genes for resistance to the antibiotic ampicillin. As a control, a tube of bacteria without pAMP shows growth cannot occur in the presence of ampicillin and that the untransformed E.coli are not already ampicillin resistant.

**Agarose Gel Electrophresis:**

Gel electrophoresis separates macromolecules such as DNA, RNA, or protein based on the rate at which they migrate through a gel under the influence of an electric field (Atherly, 1999). First DNA is digested with different restriction endonucleases in a separate tube to create DNA restriction fragments. These charged fragments are placed in wells near one end of a thin slab of polymeric gel. The gel is supported by glass plates and bathed in an aqueous solution (buffer). Electrodes are attached to both ends of the

gel, and an electric current is applied. The DNA fragments migrate through the gel matrix toward the electrode of opposite charge at a rate determined mostly by the molecule's charge and size (Campbell, 2000). For nucleic acids, the rate of migration- how far a molecule travels while the current is on- is inversely proportional to molecular size. Nucleic acids carry negative charges on their phosphate groups proportional to their length. Smaller DNA fragments move through the gel more rapidly than large fragments because the matrixes in the gel impede longer fragments more than it does shorter ones. The gel is stained with ethidium bromide to create fluorescent bands when exposed to ultraviolet light. The size of the unknown fragments can be determined by comparing them with the migration patterns of fragments with known molecular weight.

**Polymerase Chain Reaction (PCR):**

The polymerase chain reaction is a method for amplifying a specific segment of DNA. The starting material for PCR is a solution of double-stranded DNA containing the nucleotide sequence that is targeted for copying, a heat resistant type of DNA polymerase (which catalyzes the reaction), a supply of all four nucleotides (for assembly of new DNA), and primers (Mullis, 1994).

The steps for PCR are as follows: 1. The double-stranded DNA to be amplified is briefly heated to separate the two strands. 2. The DNA is then cooled to allow the synthetic primers to hydrogen bond to each strand. The primers define the ends of the sequences to be amplified. 3. Two complementary strands are synthesized with DNA polymerase by adding nucleotides to the 3' ends of the primers, using the longer DNA strands as templates (Atherly, 1999). The solution is then heated again, starting another

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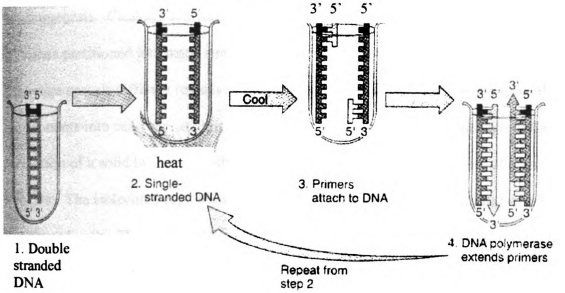
cycle of strand separation, primer binding, and DNA synthesis. After 20 cycles there are over a million copies of DNA and after 30 cycles over a billion copies. The final number of DNA copies can be calculated using 2n, where n is the number of cycles (Atherly, 1999).

Figure 2: The Polymerase Chain Reaction




**Developmental Biology**

Fertilization initiates physical and molecular changes in the egg cell. As a sperm migrates to the follicle cells to the zona pellucida it discharges hydrolytic molecules to induce an acrosomal reaction, which allows the sperm to penetrate the zona pelucida (Campbell, 2000). As soon as one sperm enters the egg, two reactions occur to prevent any additional sperm from entering. In the fast block to polyspermy the egg plasma membrane becomes depolarized preventing its fusion with additional sperm, whereas an unfertilized egg's cytoplasm is negatively charged relative to the outside. Release of positively charged calcium ions from the egg's endoplasmic reticulum during the cortical

reaction causes a hardened fertilization membrane to form, which is called a slow block to polyspermy.

Early embryonic development charts the course from a single fertilized egg to an organism made of many differentiated cells organized into specialized tissues and organs. It includes cleavage of the fertilized egg to form a blastula, gastrulation, and organogenesis. Cleavage is a succession of rapid cell divisions during which the embryo becomes partitioned into many small cells, called blastomeres, without a growth phase. Cleavage parcels different regions of the cytoplasm that contain different cytoplasmic components into cells and sets the stage for later development. Cleavage leads to the formation of a solid ball of cells, the morula, and then usually a hollow ball of cells, the blastula. The isolecithal eggs of most invertebrates and simple chordates have evenly distributed yolk. They undergo holoblastic cleavage, which involves division of the entire egg. In the moderately telolecithal eggs of amphibians, a concentration of yolk at the vegetal pole slows cleavage so that only a few large cells form there compared to a large number of smaller cells at the animal pole. The highly telolecithal eggs of reptiles and birds, with a large concentration of yolk at one end, undergo meroblastic cleavage, which is restricted to the blastodisc (Solomon, 2000).

In gastrulation, three germ layers- the outer ectoderm, the middle mesoderm, and inner endoderm that lines the embryonic digestive track form. Each layer gives rise to specific structures. In the sea urchin, cells from the blastula wall invaginate and eventually meet the opposite wall. The new cavity formed, the precursser of the digestive tube, is the archenteron, which has an opening to the exterior, the blastopore. In amphibians, the yolk cells obstruct invagination at the vegetal pole. Therefore, cells from

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the animal pole move down over the yolk cells and invaginate to form the dorsal lip of the blastopore. In the bird, invagination occurs at the primitive streak, and no archenteron forms.

In terrestrial vertebrates, the primary germ layers give rise to four extraembryonic membranes. The chorion surrounds the embryo and the other membranes. The amnion encloses the embryo in a fluid-filled amniotic cavity, which keeps it moist and acts as a shock absorber. The yolk-sac membrane, enclosing a small fluid-filled cavity, is the site of early formation of blood cells. The allantois forms blood vessels that connect the embryo with the placenta through the umbilical cord. These membranes in humans are homologous to those of reptiles and birds.

In organogenesis, the organs of the animal body develop from the three embryonic germ layers. One of the earliest events of organogenesis is the formation of the nervous system by induction of the dorsal mesoderm along the roof of the archenteron (Campbell, 2000). In addition, ectoderm, which is above the notochord, forms a neural tube from which will develop the central nervous system. Mesoderm along the sides of the notochord condenses into somites, which give rise to the vertebrae and skeletal muscles. The brain and spinal cord develop from the neural tube.

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Table 1. Sequ

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## IMPLEMENTATION OF UNIT

One main goal of the genetics unit was to teach molecular genetics and developmental biology through a variety of procedures used in major research laboratories. Pre-laboratory discussions, problem-solving activities, simulations, demonstration of proper techniques, and post-laboratory discussions are essential instructional elements to ensure that students have successful experiences in genetics. My aim was to enhance students' excitement in genetics by alternating the information and activities of biology, so students connect concepts discussed in lecture and discovered in the laboratory. A series of manipulative models, current biotechnology laboratories and developmental model organisms were researched during the summer of 2000 at MSU. Taken together, students learn about microbiology, molecular biology, genetic engineering, and embryology.

Table 1: Sequence of topics in unit

| Topics Covered In Lecture | Corresponding Activities |
|---|---|
| DNA Structure | Breakfast DNA<br>Y Chromosome Demonstration |
| DNA Replication | Manipulating DNA Replication |
| Bacterial Transformation | Bacterial Transformation Prelaboratory<br>Bacterial Transformation Laboratory |
| Gel Electrophoresis | Gel Electrophoresis Prelaboratory<br>Gel Electrophoresis Laboratory |
| Polymerase Chain Reaction and DNA fingerprinting | All You Have ALU Laboratory |
| Vertebrate Development | Zebrafish Laboratory |

**DNA Structure:**

I restructured my lecture-dominated unit covering CH 16: The Molecular Basis of Inheritance in the students' Campbell textbook by adding food and craft materials. The unit begins with a description of the structure of DNA, which some students find difficult because of the chemistry involved. To help illustrate the structure of DNA, I modified an activity out of the American Biology Teacher journal (Byrd, 2000). Students are given cereal and pretzels to build unique models of DNA, while learning all the details and background history in "Breakfast DNA" (Appendix AI.).

First students identify their bag of goodies and are given their corresponding representation: colored Fruit Loops are nitrogen bases, Golden Grahams are deoxyribose, and pretzels are phosphates. Students are shown how to construct a simple nucleotide by connecting certain types of foods together. From there, the details of DNA structure are presented with the aid of an overhead transparency. Students then proceed to make a polynucleotide chain in the 5' to 3' direction, which is reinforced with the activity for DNA replication. At this point we discuss the double stranded nature of DNA and base pairing. Students are asked to create their own 15-nucleotide sequence. They are told that they can now use just the Fruit Loops (nitrogen bases to represent the deoxynucleotide). Once they record the sequence on their paper they are asked to have their neighbor bond the corresponding nucleotide using base pair rules. After they check their neighbor's double strand they are asked to illustrate the DNA replication process of their own DNA sequence.

In addition to student questions and personal observations, the written assessment of this activity was their illustration of DNA replication. By working collaboratively

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very few of my students even needed my help on their drawings. Actually, my best assessment came from student's comments during the activity. One students recognized there were broken or discolored Fruit Loops and said, " they could be mutations". The same student also noticed the orange Fruit Loops were not being used and asked if they could eat them. They were asked if the orange Fruit Loops could represent any other bases in different nucleic acids. Once they remembered RNA has uracil in place of thymine, they were allowed to eat the orange ones. The most helpful comment though, came from one of my female students who recommended that I use Honeycomb cereal for the deoxyribose next time because it had six sides for the 5 carbons and 1 oxygen, which I'm going to do next year. I was also pleased when one of my students in this class who returns two periods later as a lab assistant brought her left over baggy of snacks and showed my other lab assistant, not in AP biology, how to make DNA and replicate it using her food.

My rationale for this activity and many of the following ones is the extensive research to create a more "hands-on" interactive environment when teaching genetics in the classroom ( Byrd, 2000). Simply stated, students remember more information when they are actively participating in the lesson. I've had wonderful experience in the past using food as a tool to teach the structure of cells and the chemical bond formation during photosynthesis. In this activity, background information can be accomplished without loss of student attention because they have something to touch, taste, see, and if they want, smell. Food always gets students attention because they know they will probably get to eat it at some point. Moreover, this activity contributes to the scientific principles taught because students use the different colors and shapes to visualize the patterns that

are recognized at the molecular level by the DNA binding proteins, which are covered later (Byrd, 2000).

To make the structure of DNA even more personal, I asked for some students to volunteer to prove their manhood (only the presence of their Y chromosome). I researched and collaborated with Dan O'Mally of the MSU Cytogenetics Laboratory and came across a wonderful fluorescent stain called Quinacrine mustard. This laboratory was done only as a quick demonstration due to a time constraint placed by the other labs that they were performing (Appendix AII). Although the procedure is very simple, the technology needed to view the chromosome is very expensive. Flourescent microscopes cost tens of thousands of dollars and require extensive training to focus. Fortunately, students were able to view the pre-focussed chromosomes from the computer monitor and microscope. In the future I hope to have more students participate in this laboratory, especially some girls to use as a control.

**DNA Replication:**

This year I introduced DNA replication with a ziplock baggy full of colorful pipe cleaners, stickers, and an assortment of beads in an activity called "Manipulating DNA Replication" (Appendix AIII). This way, students could build the model as I explained the process of DNA replication. In summary, two pipe cleaners are used to represent parent DNA molecule. A red pipe cleaner for one of the original template strands is twisted around a green one for the other template strand. Each of the four ends are labeled with the antiparallel 5'$PO_4$- and 3'OH. Students thread a purple pony bead to represent DNA helicase and correspondingly unwind the two strands. I use word

association phrases like "Helicase hacks"the hydrogen bonds between the double helix to make two strands" to help students remember the function of the enzymes involved. When I teach them about lagging strand synthesis I use the phase "Polymerase pastes" nucleotides alongside the template. Students learn about the requirements of polymerase III ( pink pony bead) by adding a small RNA primer (white pipe cleaner) at the free 3' OH group and an additional shiny red pipe cleaner (leading strand) is wound the same template strand continuously towards the replication fork (helicase bead). Lagging strand synthesis is modeled by discontinuously priming and adding smaller green pipe cleaners (Okazaki fragments) in the opposite direction of the replication fork, which follows the overall rule of 5'→ 3' synthesis. Lastly, a lime bead (symbolizing DNA ligase) "links" the fragments together.

I extended the DNA replication model by showing students a picture of leading and lagging strand synthesis occurring simultaneously. Afterwards, I had them loop their pipe cleaners and trace the direction of synthesis using their 5' and 3' labeled ends. To assess their short-term understanding and recall I had them discus in small groups a few written questions from a study guide. As reinforcement the following day, I incorporated some animation from the Biology Place website into their class discussion. This paid subscription to the biology.com web site is a powerful new tool that facilitates the learning process. Students can view animations to facilitate learning about difficult scientific processes such as DNA structure, replication, etc (Bodzin, No date). I was also able to assess their understanding of the process by watching students use the DNA replication simulations. Most students were able to correctly build an identical DNA molecule from all the "materials" provided on their screen.

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**DNA Assimilation / Bacterial Transformation:**

The basic lesson plan of this section required about 4 days of instruction. First, I started with a lecture and discussion on human use for DNA transformation technology; the Griffith experiment, which showed that rough-nonvirulent *Streptococcus pneumoniae* became virulent (and smooth) when mixed with heat-killed virulent bacteria; and the difference between natural and artificial transformation. Next, students went to the computer lab to begin their pre-laboratory assignment called "Bacterial Transformation Prelaboratory" using the Biology Place website (Appendix AIV). The website http://www.biology.com has all 12 of the required Advanced Placement Laboratories on their "lab-bench". It gives students a good summary of key concepts, lab technique hints, and experimental design. Next, students proceeded into the authentic lab " Experiment 1: Bacterial Transformation", which relates to the core of pharmaceutical research: to be one step ahead of the ever-evolving pathogens that infect us daily. (Appendix AV).

This microbiology laboratory invites students to use antibiotic-resistant plasmids to transform *Escherichia coli*. Equipped with sterile pipettes, students transfer ampicillin sensitive E. coli cells to cold $CaCl_2$ solution. Then pAMP is added only to the experimental cells. Both control and experimental bacteria are heat shocked at which time some of the competent cells take up the pAMP and are transformed. Students then spread the treated bacterial cells on LB agar plates, some with ampicillin and some without. They wrap their plates, incubate them overnight, and wash their hands. The next day they record the number of bacterial colonies on both control and experimental plates. Students compare and contrast the number of colonies on certain pairs of plates to determine that the cells are viable, the antibiotic is active, only transformed cells grow in

the presence of ampicillin, and transformation is a very rare event. Moreover, they determine the transformation efficiency of the experiment and describe what factors might influence it.

Overall the lab was successful with only one group not getting growth on a plate. Students also did very well on the laboratory analysis questions. The class average was 17.8 out of 19 points (Appendix CI). Explanation of discrepancy from ideal data was discussed and recorded as probably due to incorrect temperatures in the beakers of water. In the future I will use water baths instead of hot plates and beakers. Some students had a few points deducted from their lab for not including units in their transformation efficiency. A few points were also deducted for omitting an explanation of the effect of factors that might influence transformation efficiency. One group didn't convey that they understood one of the results of comparing LB/ Amp- and LB- plates were to show that the antibiotic was active. However, they did get credit for saying, "normal E.coli can't grow on ampicillin"

Although sterile technique and safety issues concerning live pathogens was discussed before the lab, one student returned to class after school very concerned that she had ingested some antibiotic resistant E.coli. She said that she accidentally stuck the same pen she used to count the colonies in her mouth out of habit. She worriedly asked if she was going to be all right, especially after another student in the class had been out for a week with food poisoning. After being a little panicked I asked her if she actually touched her pen to the bacteria. Luckily, she followed the procedure and only contacted the glass petri dish with her pen, so I didn't need to call an ambulance!

**Agarose Gel Electrophoresis:**

A short dry laboratory called "DNA Scissors: Introduction to Restriction Enzymes" was used to begin the next experiment. In this paper/scissors activity, students are introduced to restriction enzymes and simulate the activity of restriction enzymes with scissors. They are also introduced to restriction maps and asked to make predictions. After a brief discussion of "sticky ends", students are given time to begin the interactive pre-laboratory for the gel electrophoresis lab using Biology.com (Appendix AVI). In addition, students are involved with demonstrations of biotechnology techniques, such as loading gels with a micropipette. The demonstration is made visible to the class by gathering everyone to the front of the room and modeling the technique with a few students. Then each group of students are given a sample gel and colored dye kit to practice before using the expensive biotechnology kits.

The "authentic" lab is done as the first part of an all day field trip to Kedzie Hall on the campus of Michigan State University with special accommodations made by Dr. Merle Heidemann. Electrophoresis is the laboratory technique used to separate DNA fragments based on size. Its basis lies in the fact that DNA's negative charge when suspended in a conducting medium will migrate in an electric field. During Experiment 3: Gel Electrophoresis Laboratory, students prepare a sample of undigested DNA for use as a control (Appendix AVII). In addition they prepare a sample of phage lambda DNA digested with EcoRI endonuclease. Students prepare a third sample of DNA digested with a standard restriction enzyme (HindIII endonuclease). The fragment sizes produced by Hind III are known and provide a standard against which fragment sizes produced by EcoRI endonuclease can be compared. Lab groups load each of their three DNA samples

into individual wells. A tracking dye is added to the sample to show the leading edge of DNA migration. Students separate the sample fragments using gel electrophoresis. The electrophoresis apparatus is turned off when the tracking dye nears the end of the gel to prevent the running off of the actual DNA. The gels are immersed in a dye that allows the fragments to be observed. In this case Carolina Blue is used and easily destained with water. Using semi-log graph paper, students graph a standard curve using the observed migration distances and known fragment sizes for the HindIII standard endonuclease. Since migration distance is inversely proportional to fragment size, plotting migration distance against the log of the number of base pairs produces a straight line. Using the best-fit line, students can then interpolate the size of each EcoRI fragment produced.

Overall, students seemed to understand the main objectives of the lab with an average score of 16.2 out of 19 (Appendix CI). In addition, most students' gels were readable. There were a few misconceptions found in their lab analysis. A few students drew the digested DNA fragments in different wells after separation by electrophoresis, instead of bands in the same lane. The same students described the affect of voltage in electrophoresis as " if greater, the length of fragments would appear to be more, if less, the length of fragments would appear to be less". For the amount of DNA used they said " more bands would show up and possibly confuse the data/ readings if more DNA were used". When the labs were returned we discussed how increased voltage will increase the rate of migration and increased amounts of DNA will increase the intensities of the fragments after staining. For the most part, the class understood the principles of electrophoresis and restriction analysis to answer the questions on the laboratory and correct their peers' misunderstandings.

The rationale for conducting this experiment was to solidifiy students' understanding of these topics by integrating abstract concepts into a concrete experience. Using newly acquired genetic knowledge in a real world context helps students understand the genetic concepts and provides motivation for their learning (Munn, 1999). Many of the techniques that are fundamental to genome research and biotechnology, such as restriction fragment analysis and DNA sequencing can be carried out in a high school classroom. The complexity of the lab does not fit into the high school class periods and the benefits of taking a group to a university are enormous. Ironically, when students were asked to comment on the laboratory one wrote, " we're missing school". Fortunately, another student wrote " I learned more about research at MSU and got a chance to use and look at a lot of expensive research tools".

**Polymerase Chain Reaction (PCR):**

As with the Gel Electrophoresis Lab, the third authentic laboratory called "All You Have ALU" was performed at Michigan State University (Appendix AVIII). This lab could not have been accomplished with out the generous use of equipment and materials by John Gerlach and collaboration with Roger Herr of the Medical Technology Department. During my research time at MSU, I spent several days learning the basic theory and adapting them for high school student use. Roger Herr also gave me an opportunity to practice the lab procedure on my peers using the thermo-cycler, 1kb DNA Ladder standard marker, gel electrophoresis apparatus, ethidium bromide, and digital florescent camera. His help has made this lab one of my best learning and teaching experiences.

To introduce students to the laboratory I had them visit the Cold Spring Harbor DNA learning center at http://vector.cshl.org/Shockwave/pcranwhole.html. They were asked to read the introduction on polymerase chain reaction and view the animated amplification process of PCR. I reviewed the information on polymerase chain reaction with a few PowerPoint slides and had them color code and label the steps of PCR. Students were given the laboratory exercises to read on the hour-long bus ride from Grand Blanc to East Lansing. During that time I migrated from group to group on the bus to discuss the labs and ask if there were any questions. I also delegated specific tasks to different lab groups.

In summary, the All You Have Alu lab uses the polymerase chain reaction to amplify a short nucleotide sequence from human chromosome 16. The object is to create a personal DNA fingerprint that shows the presence or absence of the Alu transposable DNA sequence on each chromosome at the PV92 locus. The source of DNA for this procedure is a sample of squamous cells obtained from student cheek cells. The students collect their cells using saline mouthwash, separate them by centrifugation, and resuspend the cells in Chelex. Students boil the samples to lyse the cells and liberate the chromosomal DNA. The Chelex binds metal ions, released by the cells that inhibit the PCR reaction. Each student combines their DNA sample in clear supernatant with a buffered solution of heat-stable Taq polymerase, oligonucleotide primers, the four deoxynucleotides, and the cofactor magnesium chloride.

I demonstrated the correct use of the three different micropipettes and organized separate groups to use the microcentrifuge because only 8-10 samples could be run at a time. In the future, I would borrow more microcentrifuges to reduce the 40-minute wait

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time between the first and last group. Fortunately, those students who were done first could check on the gel electrophoresis lab and clean up. We did have a quick show of the effects of an unbalanced centrifuge, but no harm was done. Considering the number of steps in the procedure, rotation of the steps between groups, variety and of new lab equipment and materials, and keeping an eye on their other experiment, I think this group did a wonderful job.

Roger Herr generously demonstrated the next part of the lab procedure. He placed each student's labeled PCR mixture in the DNA thermal-cycler and programmed the machine to run 30 cycles. Each cycle consists of 30 seconds at 94°C to separate the DNA strands, 30 seconds at 58 °C to bind the primers and 30 seconds at 72°C to extend the deoxynucleotides. Moreover, he loaded the amplified products into the agarose gel because of the toxicity of Ethidium bromide stain.

The only assessment in this lab was the effort and technique used during the experiment and the written group analysis of the photographed results. I divided the class into two groups by the coded numbers on their samples. Each group was to determine the resulting DNA fingerprints on their photo and share it with the class. The resulting bands of this lab were crystal clear compared to the Carolina Blue stained gels in the Gel Electrophoresis lab. 15 of the 18 samples could be easily analyzed. Unfortunately, three student samples were impossible to decipher. One student pippetted her sample incorrectly and couldn't retrieve enough of it to show on the gel. The other two students' samples were lost in the boiling water baths because they did not close their safety locks. We heard a couple sample tubes pop loudly, but those two tipped over in the water before we could save them. Of the 15 readable samples the class determined 11 students had a

clear band which appeared to be 550 base pairs, which is homozygous negative for the ALU allele; 3 samples had a band appear at 850 base pairs, which is homozygous positive for both ALU alleles; and 1 student had a band at both the 550 bp and 850 bp, which is heterozygous for the ALU alleles.

One of my rationales for doing this laboratory was because "significant learning takes place when the subject matter is relevant to the personal interests of the student" (Kearsley, 2001). In this lab students were analyzing their own DNA from their own cheek cells. Moreover, the rapid accumulation of genetic information generated by the Human Genome Project and related research has greatly increased student attention of genetic issues. By collaborating with real scientists in real research labs, my students have the opportunity to share the excitement of the field of research and hopefully be inspired to pursue some scientific research themselves or at least make educated decisions related to genetic public policy. In addition,

> *"the involvement of scientists benefits the science community directly by helping to produce a more scientifically literate population, which affects how the public perceives and accepts scientific endeavors. It also helps the university because the political and financial support, the students, the future jobs of many of the students-and ultimately, even our research problems-come from this world, not from within the university"* (Munn, 1999).

Last but not least, our school district career technology coordinator, Gary Towers, agreed to support the field trip next year with technology education funds.

**Developmental Biology**

As a model organism for vertebrate development, I chose the *zebrafish (Danio rerio)* for students to observe during the "Zebrafish Laboratory" (Appendix AIX). These

tropical freshwater fish are easy to maintain, prolific breeders, and have completely transparent embryos. Because the embryo is transparent, students can follow the development during cleavage, blastulation, gastrulation, neuralation, and can clearly see the beating heart. Students helped collect embryos from the bottom of the tank by using a rubber siphon. Then they carefully pipetted out the eggs from all the debris into a beaker full of tank water. Individual lab groups transferred several of the eggs into their own petri dish and then to a dissecting microscope. Students were to observe each egg and try to determine the stage of development. To take a closer look at the embryos students were shown how to make a coverslip bridge and view their embryos under a light microscope. In addition, they needed to sketch their embryos and record the times of their observations. Later, students needed to describe in their laboratory handout the features of each type of embryo they observed and predict the age by comparing them with their textbook and other references. Groups of students were very generous in sharing their zebrafish at different stages of development with other groups. In this way, most of the stages were identified. The later stages of development were by far the most interesting to the students. They were just fascinated by watching the little embryos flop their tails and bodies around in the egg. Some students watched the zebrafish embryo hearts beating for about ten minutes. Even the principal of our school was amazed as he peered into the microscope at a living and developing embryo.

**Assessment Tools**

The assessment tools and methods used for this unit included a pretest; class discussions; the lab drawings, labels, interpretation, and assessment questions; an essay,

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and posttest. All assessment tools can be found in Appendix B. The pretest and post-tests were the same except for the DNA structure and replication tests. All tests were multiple choice, except the gel electrophoresis and PCR tests, which had some short answer questions. The AP Biology essay question asked students to name and describe the origin, function, and mechanism of operation of the four extraembryonic membranes of a bird and to briefly describe one variation between these membranes in a bird and those in a human in either development or function. Students' essays were graded by the AP College Board 10-point rubric for this topic and were given an extra point for illustrations.

In addition to my assessments of the students, I asked students to evaluate the unit by completing a survey (Appendix BI). The survey asked them to rate the laboratory experiences, classroom activities, and the field trip. For example, students were asked to rate the classroom simulations of laboratory techniques in terms of helping them understand biotechnology concepts taught in this course. Evaluation was based on a qualitative scale ranging from excellent to unsatisfactory.

## EVALUATION

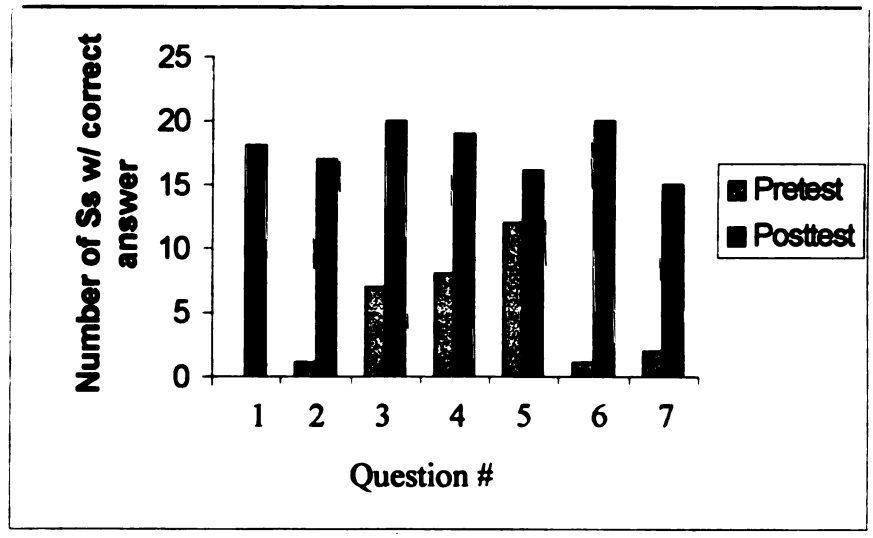
Although student understanding of this unit was facilitated by essays, class discussions, laboratory exercises, and manipulative models, the evaluation process was facilitated primarily by the pre and post-tests. The six main topics evaluated include DNA structure, DNA replication, bacterial transformation, gel electrophoresis, polymerase chain reaction, and developmental biology. Each of the students was given a varying number of multiple choice questions before and after the unit. In addition, both the gel electrophoresis and PCR portion of the evaluation process included several short answer pre and posttest responses. The test questions were targeted to weak areas discovered during the first year's AP Biology students or topics that had not been included due to a lack of time. These pretest and post-test questions can be found in Appendix B. The questions asked were chosen based on topics that had not been included during the prior year or had previously shown low student comprehension.

The null hypothesis is that there will be no difference between the pre and post-test scores of the students. The individual student test results are shown in Appendix CII. The null hypothesis was rejected at a significance level below 0.001 using the t-test for all pre and post-test data. Stated another way, there is a 99% probability that the observed test gains are due to something besides chance. The t-test probabilities and inverses of the t-distributions are located in Appendix CIII. In addition, the class's average pretest percentage for the unit was 16.9%. After the lessons were taught with the new teaching activities, the average students score on the same questions rose to 77.2%.

These statistically significant results are included in Appendix CIII. In general, this year's student's scores are better in comparison to the first year's scores.

The first subject specific set of evaluation data is from the DNA structure pre and post-tests results from questions 1-4 on Table 5 (Appendix CV). The objectives analyzed on these tests included students being able to identify the three components of a nucleotide, distinguish between the nitrogen bases, describe base pair rules explain what kind of chemical bonds connect the nucleotides of each strand, and explain what type of bond holds the two strands together. The pre and post-tests were identical with a few exceptions. One difference in the post-test included questions where students had to label the correct nitrogen base to its complementary base pair. These questions only had the purine and pyrimidine shapes with the number of hydrogen bonds (questions13-16). See Appendix BII for the complete tests. The average number of questions students answered correctly on the pretest was 0.8 out of a possible 4 compared to the 3.5 out of 4 on the posttest (Appendix CII). Over half the class received perfect scores on the post-test. The greatest improvement was seen on question number 1 (Figure 3). Question 1 asked students to

**Figure 3: DNA Structure & Replication Test Results**



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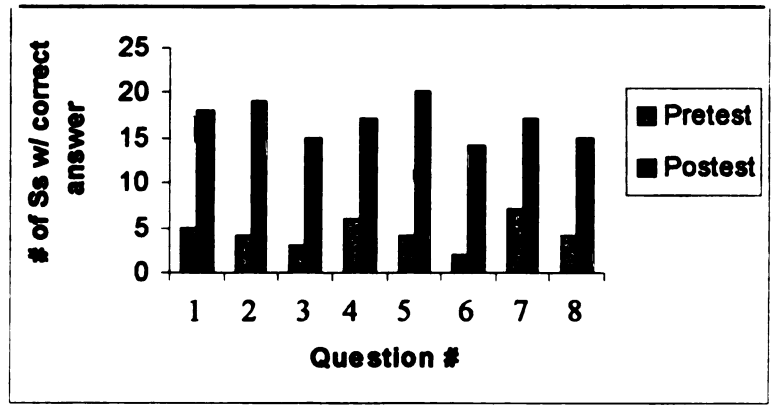
identify a chemical group that, together with a sugar and a nitrogen base, makes up a nucleotide. No students had the correct answer on the pretest, whereas 18 students correctly answered phosphate group on their posttest. Almost half the class were able to identify a 5' carbon of deoxyribose on their pretest which contributed to the lowest gain, even though all but one student got it on the posttest.

The objectives targeted on the DNA replication portion of the tests, questions 5-7 on Table 5, focussed on students being able to describe the process of DNA replication, explain the role of enzymes, and explain the Meselson-Stal semiconservative model of replication (Appendix BII). Students showed an improvement in their performance on the pretest by increasing from an average of 25% to an average of 80% on the post-test (Appendix CIV). The greatest improvement was observed on students understanding of the type of replication DNA undergoes. Only 0.5% of the students answered question 6 correctly on the pretest, whereas 95 % of the students recalled that DNA replication was semi-conservative on the posttest (Figure 3). The data on the pretest showed that half of the students, who had gotten this question wrong, had the misconception that DNA replication was conservative. The smallest difference between pre and posttest scores came from number 5, which asked students to identify (on a figure) what is most likely to be broken during replication (Figure 3). 12 students on the pretest and 16 on the posttest were able to determine that the dotted lines between the bases were the bonds broken, but students did not recognize them to be hydrogen bonds (question 2 in Appendix BII). Overall, half the class received perfect scores on the DNA replication portion of their posttest that matched the pretest. In addition to the questions asked on the pretest, several other questions regarding DNA replication were assessed on the posttest. These

included students' ability to distinguish the processes that occur during replication, the composition of primers needed to initiate DNA replication, and which enzymes are necessary for DNA replication.

The pre and posttest questions for the Transformation unit were the same for both tests. These test scores were used to assess student's understanding of natural genetic recombination in bacteria, the composition of a bacteriophage, the structure of a bacterial chromosome, the process of transformation, types of vectors used in recombinant DNA technology, the structure of a plasmid and characteristics of plasmids. The whole class improved their performance on the eight pretest questions by an average of five points; each question was worth one point (Appendix CII). Eight students out of 20 received a perfect score on the transformation posttest (Appendix CII). The greatest amount of improvement was on question number 5, which every student answered correctly on the posttest (Appendix CVI). This question asks students to understand that DNA, ribosomes, cell membranes, and enzymes are all found in prokaryotic cells, whereas nuclear envelopes are not (Appendix BIII). The question with the smallest difference from pre to post test was question number 7, which asks students to understand that plasmids can be used as a DNA vector (Figure 4).

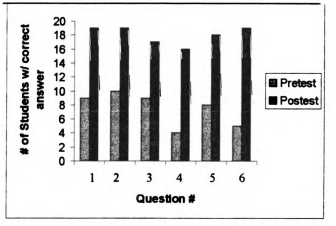
**Figure 4: Bacterial Transformation Pre & Post-test Results**



This difference is partially due to the fact that students scored the highest on this question during the pretest. Oddly, the three students who got this question wrong all said that a plasmid is a type of bacteriophage.

The objectives of the Electrophoresis portion of the unit focussed on students being able to describe how restriction enzymes and gel electrophoresis are used to isolate DNA fragments, and interpret results from gel electrophoresis semi-log graphs. The first type of data collected was in the form of students responses to multiple choice questions, whereas the second type of data collected was from student's responses to short answer questions (Appendix BIV). On the multiple choice tests, Students showed an improvement in their performance on the three question pretest by increasing from an average of 37.5% to an average of 90% on the posttest (Appendix CIV). The greatest improvement was observed on students understanding of the fundamentals of nucleic acid separation of gel electrophoresis. Only 5 of the students answered question 6 correctly on the pretest, whereas 19 of the students remembered that nucleic acids are separated on the basis of differences in their size (Appendix CVII). This concept was emphasized repeatedly on the pre laboratory simulation, class discussions, and the laboratory exercise. The smallest difference between pre and posttest scores came from question 3 (Figure 5).

**Figure 5: Electrophoresis Pre & Post-test Results**




This question asked students to use the semi-log graph results from a gel electrophoresis

procedure to approximate how far a DNA fragment with 3000 base pairs travels on a gel. 9 students on the pretest and 17 on the posttest were able to interpolate the data and determine the DNA fragment migrated 2.6cm (Appendix BIV).

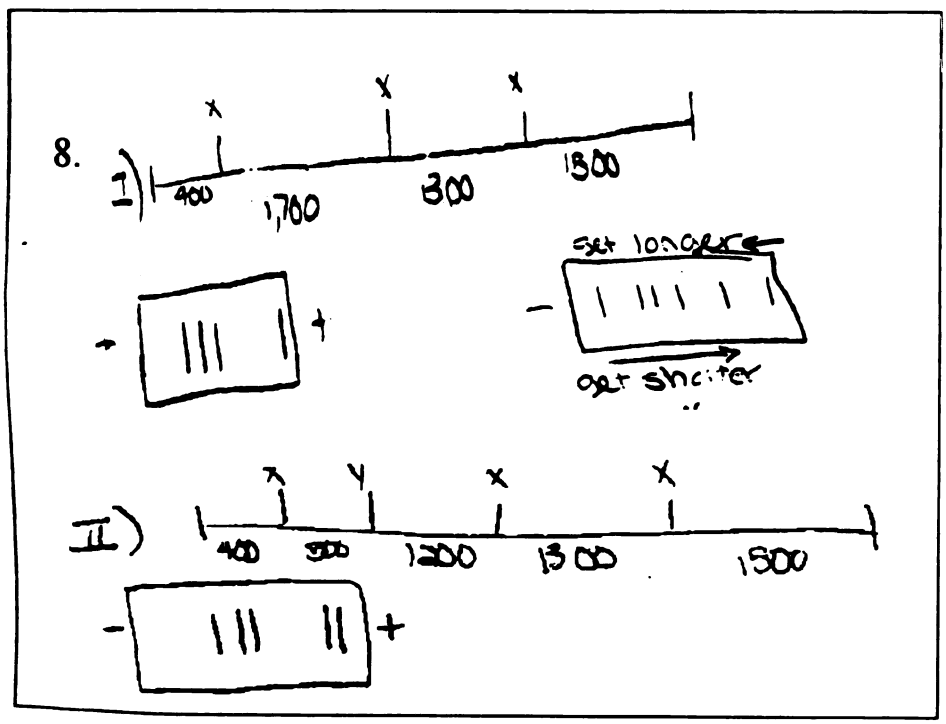
The second half of the Electrophoresis pre and post test included two short answer questions that were taken from Advanced Placement College Board essays on previously released tests . Each question was, as objectively as possible, evaluated using the assessment rubric in Appendix BIV. Some samples of students' responses are included in this evaluation. All names of the test subjects have been changed to protect their privacy, as was stated on the parental and student consent forms approved by the University Committee on Research Involving Human Subjects.

Question 7 asks students to "Explain how the principles of gel electrophoresis allow for the separation of DNA fragments" (Appendix BIV). Overall, only one student received any points for her responses to this question, but she was also allowed to take it home because she was not present when the rest of the class took the pretest (Appendix CII). Most students just left the question blank, few students responded with "I don't know", and only one student said, "small monkeys run with it". As can be inferred by these results, students have had no prior experience with gel electrophoresis. On the post-test another student answered the question by writing, "Gel electrophoresis separates DNA fragments through the following process: DNA has a negative charge and consequently will travel towards the positive electrode in an electrophoresis through the gel. Because of basic laws of particle physics and common sense, it follows that the shorter fragments will travel more quickly than the larger fragments. If this process is stopped before all the segments of DNA reach the end of the gel, one can see the various

strands of DNA in their progression to the (+) end and determine the relative lengths of these strands, thus separating them based on their lengths." In accordance with the grading rubric (Appendix BIV), this response received 3 points compared to the 0 points he earned on the pretest (Appendix CII). Some of the students included the need for there to be a current to carry the negatively charged DNA, but no students mentioned the need for a buffer to carry the current across the gel. Moreover, students overlooked the necessity of a stain to view the DNA bands in the post-test.

Question 8 asks the students to "Describe the results you would expect from electrophoretic separation of fragments from the following treatments of the DNA segment above (see Appendix BIV). Assume that the digestion occurred under appropriate conditions and went to completion. I. DNA digested with only enzyme X. II. DNA digested with enzyme X and enzyme Y combined. All students scored a 0 on the pretest, even the student who took it home.

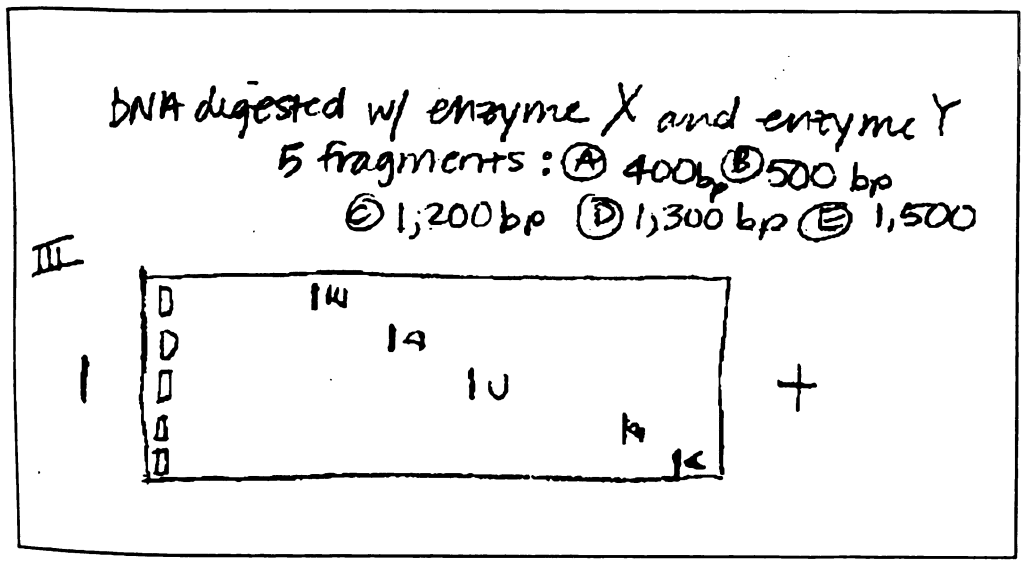
**Figure 6: Student work form post-test question 8**



A senior female, answered question 8 part I by writing, " I would expect there to be 4 different fragment lengths. The shortest 400, then 1300, then 1500, and then 1700." On question 8 part III, she responded by " I would expect there to be 5 different fragment lengths. The shortest 400, then 500, then 1200, the 1300, then 1500 (longest)". The student also included the following illustrations (Figure 4) for better explanation, as was requested.

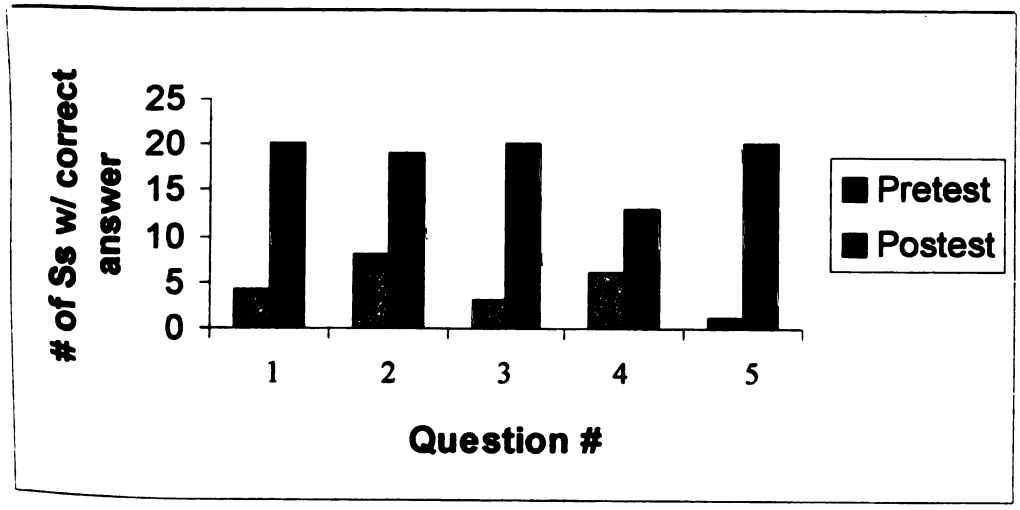
Although most students could describe the DNA digestion and resulting sequence of bands on the gel, a couple students drew misleading illustrations (Figure 7). Notice that the DNA fragments are in different lanes, which gives the misconception that the DNA was loaded into separate wells instead of the same. This response and the same for part II, earned this student and a few other students 2 points out of the possible 4. Table 2 shows that the overall improvement in the scores was 3.1 points out of 7 (Appendix CII).

**Figure 7: Second student response to post-test question 8**



The Polymerase Chain Reaction pre and posttests were used to assess student's understanding of the following DNA Technology concepts: how restriction fragment length polymorphism analysis and PCR can be applied; how the creation of sticky ends by PCR restriction enzymes is useful in producing recombinant DNA; and the purpose of PCR, and the steps and materials involved. Again, the first type of data collected was in the form of students' responses to multiple choice questions and the second type of data collected was from student's responses to short answer questions. On the multiple choice test questions, the average score on the pretest was 1.1 of the possible 5 points compared to the 4.6 out of 5 on the posttest (Appendix CII). There were 12 students who received a perfect score on the posttest (Appendix CII). The greatest amount of improvement was on question number 5, which asks students to recognize the requirements of restriction fragment length polymorphism to determine parentage (Figure 8). 100 percent of the students recalled that every band present in a child would be expected to be present in at

**Figure 8: PCR Multiple Choice Pre and Post-test Results**



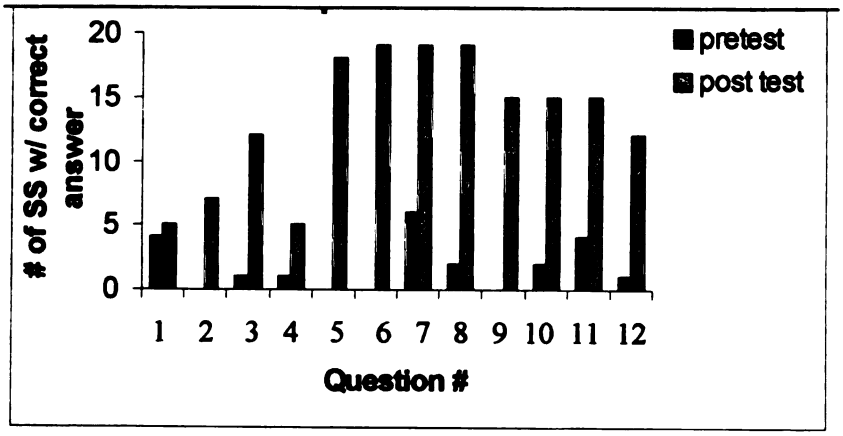
least one of the true parents compared to only 1 of the students on the pretest (Appendix CVIII). The posttest question 1 and 3 also showed that all students could master this information visually by interpreting the "family's DNA material" (Appendix BV) . Even after doing the "All you have ALU" laboratory at MSU, 30% of the students did not understand that PCR technique uses heat resistant DNA polymerase . Most of these students chose the incorrect answer involving DNA ligase. Unfortunately, the questions on the AP tests can be very specific.

The second half of the PCR pre and posttest included a two-part short answer question. In the same way, each question was, as objectively as possible, evaluated using the assessment rubric in Appendix BV. Questions 6 and 7 asks students to describe "What the PCR technique is and what the major steps are" Not a single student received any points on the pretest, but these responses were some of my most entertaining. Some of the student's responses for what PCR is and why it is valuable were "people chasing rabbits", "because without it, how would we catch the rabbits"; "CPR in a different order" , "to save lives". As with the other short answer pretest questions, most of the students just left the answer space blank because they probably never heard of it before. A male student answered the question on the posttest by writing, "so that we can isolate parts of DNA and identify them". He described the steps of PCR as "1). Denature DNA at high temperatures, 2). Allow plasmid attaching, 3) Therefore DNA can follow its due process". These responses earned him 0.5, 1, 0.5, and 0 pts respectively, for a total of two points out of four (Appendix CII). Another student received full credit for saying " PCR technique can amplify or make the supply of a particular gene or protein more abundant. It can do this quickly" She went on to describe the steps of PCR with "1) Heat

the strands at 94°C to denature the strands, 2. Cool to 58°C so the primer can be added to the separate strands, 3) raise the temp to 72°C so the DNA polymerase can lay down the proper bases." Other students also received full points for describing the change in temperature, (i.e. "raise, lower, raise"), instead of the actual temperatures used during the experiment. Because the pretest scores were all zeros, I did not graph the difference between pre and post-test scores, but instead organized the scores earned into Appendix CII. From this table, it is easy to see that the most common score earned was a 3 out of 4 with 8 students earning that score. The next most common score was a 4 out of 4. The total test group improved their performance on this question by 2.9 points, which was the average on the posttest (Appendix CII).

The developmental biology pre and post-test were used to assess student's mastery of the following developmental concepts: fertilization, stages of early embryogenesis, and the role of extraembryonic membranes. The average number of questions students answered correctly on the pretest was 0.9 out of a possible 12 compared to the 8 out of 12 on the posttest (Appendix CII). There was only one student who received a perfect score on the post-test. The greatest amount of

**Figure 9: Developmental Biology Pre and Post-Test Results**



improvement was on question number 6, which asks students to determine what process establishes the primary germ layers (Figure 9). 95 percent of the students recalled that germ layers are produced during gastrulation compared to the 0 percent on the pretest (Appendix CIX, question 6). The posttest showed that most students could describe the correct sequence of stages during embryogenesis and describe the major distinguishing events during fertilization and cleavage, which were heavily emphasized during the zebrafish lab. The two areas in which they were still weak were illustrating an example of embryonic induction (Appendix CIX, question 4) and the location of human fertilization (Appendix CIX, question 1). Most of the students, especially the males who misunderstood question 1, answered that fertilization occurred in the ovary. My only rationale for students missing this question is the possibility that they were thrown off track when they didn't see fallopian tube as a choice. One of the computer simulations used to help them understand fertilization involved a female reproductive track with highlighted labels where the egg was passing through, fertilized (in the fallopian tube), and eventually implanted.. In question 2, which asked students to determine what somites give rise to in mammalian embryos, 50 percent of the students mistakenly choose "the notocord" and only 32% correctly answered "muscles and vertebrate".

**Student Survey Results:**

Overall, the unit for teaching biotechnology and embryology was successful with the vast majority of students, according to survey results (Appendix CX). Specifically, the students found the Medical Technology and Genetic Testing Field Trip to MSU to be very interesting and useful to the study of biology, with over 72% of the students rating it

as excellent. 100% of the students rated the classroom simulations of laboratory techniques, such as the "Manipulating DNA Replication" activity, as excellent to very good in terms of helping them to understand biotechnology concepts taught in this class. One of my students wrote "I still have it, it's in my car!". Imagine what the world would be like if DNA models hung from every student's rear view mirror?

In addition, ninety five percent of the students rated the question on how laboratory work related to ideas discussed in class as very helpful. By far the students rated the All You Have ALU laboratory, which involved using the PCR on their own DNA, the highest in comparison to other science course laboratories. On the other hand, over 22% of students rated the Embryology of Zebrafish as only fairly good in comparison to other science course laboratories. Possibly, a few students were unsatisfied with their grade and consequently did not value some of the course components. Although one student was less than enthusiastic about biotechnology and embryology, the majority of students rated each of the components as excellent or very good.

# CONCLUSION

The rapid accumulation of genetic information generated by the Human Genome Project and related research has greatly increased public attention to genetic issues. Molecular biology is a current hot spot in research activity, which will continue reaching into every corner of life science. Molecular biology has already become a potent tool for exploring development and embryology. Developmental biologists continue to unravel the regulatory networks that tell cells where they are in an embryo and choreograph their differentiation into specific cell types during transformation of egg to organism. High school students need to be educated in DNA science, especially those interested and able to pursue a biology-related career. Therefore, biology teachers, particularly AP biology teachers, are challenged to help students become successful in understanding these difficult concepts.

The purpose of this unit was to develop and implement a teaching model for engaging students in genetics and developmental biology. The unit contains several teaching techniques to enhance students' involvement and appreciation of biology. The overarching theme encompassed by the buzzwords of educational literature (inquiry-based, authentic laboratories, process skills, and hands-on), is the value of active learning. The teaching activities developed in this unit are governed by the fundamental principle that many students learn best by doing. These new laboratories and activities give students the opportunity to "do" quantitative and qualitative science, and ensure that they get the "big picture".

The pre and post-tests focused on the concepts of DNA structure, replication, transformation, separation, amplification, and developmental biology. The data garnered from these tests statistically show a significant increase in student performance on the selected objectives. However, the source of improvement is confounded amongst the activities, pre-laboratories, laboratories, lectures, and class discussions. This means it is difficult to conclude that one method of teaching is a larger contributor to student comprehension. It is known that students need several iterations using different method of teaching. Further study is needed in the mechanisms behind student learning with regards to molecular genetics and vertebrate development before the most critical teaching technique can be elucidated.

The student survey results might provide an avenue to intensify the study of teaching techniques. Students clearly enjoyed the biotechnology laboratory "All you have ALU" at Michigan State University. This laboratory-field trip combination was very personalized using their own DNA; required deep processing skills while engaged in the lab procedure; and involved real research equipment, facilities and researchers. Interestingly, their evaluation of the different components of this unit closely correlated with many of the educational theories researched.

There were a few weaknesses in the first attempt at implementing the new teaching activities in this unit. The major problem was time; there is never enough time to teach AP biology. Even though two of the labs were done as all day field trips, we simply didn't have enough time to have students perform the Y chromosome laboratory. Instead, they watched a quick demonstration. I plan on using multiple centrifuges during the All You Have ALU laboratory in the future to ensure extra time for students to

perform the Y chromosome lab. It is important to have a female's DNA as a control next time.

One of the major strengths of the labs was the enthusiasm they generated. One student asked if he could do an independent research project using the Zebrafish. I excitedly agreed, because self-initiated learning is the most lasting and pervasive (Kearsley, 2001). He set out to test various environmental factors to see how development can be influenced and inherited. Unfortunately, we couldn't get any of the embryos to develop into fertile adults. Near the end of the school year, by chance, I came across an article detailing the conditions needed to raise the little fish. I'm planning to have students observe the embryos develop into adult fish next year, perhaps as some kind of case study project. Another benefit gained by analyzing the data for this unit was that I was able to really focus on students' rough spots during their review right before they took the AP test. Students were very interested and appreciative when I shared some of my results.

Finally, I would like to echo the words of advice from Louis Pasteur, who would challenge instructors and their students regarding laboratories to:

> "Take interest, I implore you, in sacred dwellings, which one designates by the expressive term: Laboratories. Demand that they be multiplied, that they be adorned. These are the temples of the future, temples of well being and of happiness. There it is that humanity grows greater, stronger, and better."

## APPENDIX AI.

## BREAKFAST DNA

**Instructor's Guide:**

**Time Frame:** 1 hour

**Target Group:** Advanced Placement Biology students

**Preparation of materials:** Pour each of the materials below into a plastic sandwich bag.

**Sources of materials:** any grocery store

**Problems encountered:** Students had difficulty relating the square Golden Graham cereal to the deoxyribose sugar. Replace the Golden Graham with Honeycomb cereal.

**Reference:** The American Biology Teacher, Volume 62, Number 7, September 2000

**BREAKFAST DNA**

**Instructor's Guide:**

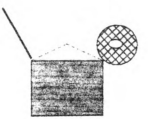
**Objective:** To use cereal & pretzels as a "hands on" tool to explain DNA structure, replication, transcription, and translation.

**Materials:**

15 red FruitLoops
15 purple FruitLoops
15 yellow FruitLoops
15 green FruitLoops
15 blue FruitLoops
cup of Alphabits cereal
cup of Golden Graham cereal
½ cup of pretzels

**DNA Structure:**

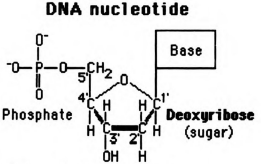
I. Nucleotide- basic backbone of the DNA molecule
   - FruitLoops – bases
   - Golden Grahams- sugars
   - Pretzels – phosphates



- **"Take one golden graham and place a blue fruitloop on the upper right hand corner".**
- **"Break the pretzel stick in half and place on of the pieces on the upper left hand corner of the golden graham"**
- ***Discuss the structure of a nucleotide* and how the graham loosely represents the sugar, the loop represents the base and the pretzel stick represents the phosphate bond.**
- **In this case the "blue" fruitloop represents thymine.**

A. The sugar is the 5-carbon sugar deoxyribose. By convention the carbons on this sugar are labeled 1' through 5'.

B. The phosphate is attached to the 5' carbon of the deoxyribose sugar.




C. The base is attached to the 1' carbon of the deoxyribose sugar.

**II. Types of nucleotides- determined by their bases**

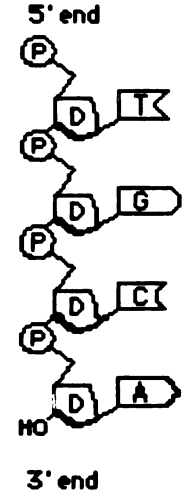
- Blue- thymine (deoxythymidine nucleotide)
- Red- adenine (deoxyadenosine nucleotide)
- Yellow- cytosine (deoxycytidine nucleotide)
- Green- guanine (deoxyguanidine nucleotide)

A. They are called nitrogenous bases because each base contains at least two nitrogen atoms.

B. There are two classes of bases
   - Pyrimidines: cytosine (C) and thymine (T)
   - Purines: adenine (A) and guanine (G)

Precede to make the other three nucleotides

Figure 5.27




**III. DNA Polynucleotide Chain**

Note: you may only slide your nucleotides, you may not pick them up!

To make a polynucleotide chain, take your deoxythmidine (blue) nucleotide and attach the pretzel stick (5' phosphate) to the bottom left corner (3' OH) of the golden graham of the deoxyguanidine (green ) nucleotide.

A. In a DNA polynucleotide chain, nucleotides are joined by phosphodiester bonds formed between the 5' carbon of one sugar and the 3' carbon of the next sugar.

Make a line of loops (any color order) 15 nucleotides long

B. 15 Nucleotide sequence: _____

**IV. Double Stranded DNA (Base pairing rules)**

A. Edwin Chargaff determined that the ratio of adenine to thymine and the ratio of guanine to cytosine were always the same in all organisms tested.
   1. The amount of A=T and the amount of C=G

B. Hydrogen bonds
   1. Two hydrogen bonds form between thymine and adenine
   2. Three hydrogen bonds form between cytosine and guanine

   Figure 16.6 Base pairing in DNA

C. The bonding of bases is complementary.
   1. The sequence of one chain dictates the sequence in the other.

D. Record your double stranded DNA molecule sequence (add H bonds)

- How could you base pair your nucleotides that are in front of you without picking them up
- Figure 16.5

E. The two complementary strands of DNA are antiparallel
   1. One strand runs 5'→ 3', whereas the other runs 3'→ 5'.

## V. DNA Replication

- After Watson and Crick proposed the double helix model of DNA, three models for DNA replication were proposed

A. Conservative Model-the two parental DNA strands are back together after replication has occurred.

B. Semiconservative Model-the two parental DNA strands separate and each of those strands then serves as a template for the synthesis of a new DNA strand.

C. Dispersive Model-the parental double helix is broken into double-stranded DNA segments act as templates for the synthesis of new double helix molecules.

D. Meselson and Stahl's experiments with E.coli showed the density of the molecules in each group matches the labeling pattern expected if DNA is replicated semiconservatively.

E. Model for DNA replication
   1. Before replication, the parent molecule has 2 complementary strands of DNA. Each base is paired by hydrogen bonding w/ its specific partner.
   2. The first step in replication is separation of the two DNA strands.
   3. Each parent "old" strand now serves a s a template that determines the order of nucleotides along "new" complementary strands. Nucleotides plug into specific sites along the template surface according to the base pairing rules.
   4. The nucleotides are connected to form the sugar-phosphate backbones of the new strands. Each DNA molecule now consists of one "old" strand and one "new strand. The two DNA molecules are identical to the one molecule with which you started.

F. Illustrate the replication of your DNA below.

**BREAKFAST DNA**

**Objective:** To use cereal & pretzels as a "hands on" tool to explain DNA structure, replication, transcription, and translation.

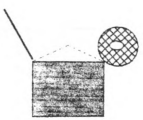
**Materials:**

15 red FruitLoops
15 purple FruitLoops
15 yellow FruitLoops
15 green FruitLoops
15 blue FruitLoops
cup of Alphabits cereal
cup of Golden Graham cereal
½ cup of pretzels

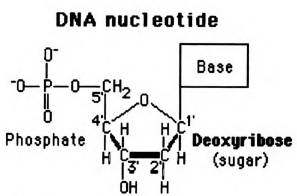
**DNA Structure:**

I. _____- basic backbone of the DNA molecule

- FruitLoops – _____
- Golden Grahams- _____
- Pretzels – _____



A. The sugar is the _____ _____. By convention the carbons on this sugar are labeled 1' through 5'.




B. The _____ is attached to the _____ of the deoxyribose sugar.

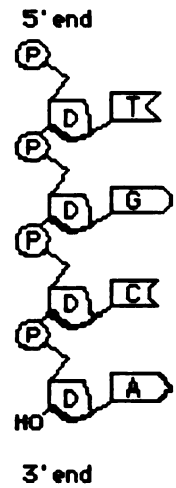
C. The _____is attached to the _____ of the deoxyribose sugar.

II. **Types of nucleotides- determined by their bases**

- Blue- _____ (_____)
- Red- _____ (_____)
- Yellow- _____ (_____)
- Green- _____ (_____)

A. They are called nitrogenous bases because _____ _____.

B. There are two classes of bases
- Pyrimidines: _____ and _____
- Purines: _____ and _____




## III. DNA Polynucleotide Chain

A. In a DNA polynucleotide chain, nucleotides are joined by _____ formed between the ___ carbon of one sugar and the ____carbon of the next sugar.

B. 15 Nucleotide sequence:

_____

## IV. Double Stranded DNA (Base pairing rules)

A. _____ determined that the ratio of adenine to thymine and the ratio of guanine to cytosine were always the same in all organisms tested.
1. _____

B. Hydrogen bonds
1. Two hydrogen bonds form between _____
2. Three hydrogen bonds form between _____

C. The bonding of bases is _____.
1. The sequence of one chain dictates the sequence in the other.

D. Record your double stranded DNA molecule sequence (add H bonds)

_____

E. The two complementary strands of DNA are antiparallel
1. _____.

## V. DNA Replication

A. _____-the two parental DNA strands are back together after replication has occurred.

B. _____-the two parental DNA strands separate and each of those strands then serves as a template for the synthesis of a new DNA strand.

C. _____-the parental double helix is broken into double-stranded DNA segments act as templates for the synthesis of new double helix molecules.

D. _____ experiments with *E.coli* showed the density of the molecules in each group matches the labeling pattern expected if DNA is replicated _____.

E. Model for DNA replication
   1. Before replication, the parent molecule has 2 complementary strands of DNA. Each base is paired by hydrogen bonding w/ its specific partner.

   2. The first step in replication is separation of the two DNA strands.

   3. Each parent "old" strand now serves a s a template that determines the order of nucleotides along "new" complementary strands. Nucleotides plug into specific sites along the template surface according to the base pairing rules.

   4. The nucleotides are connected to form the sugar-phosphate backbones of the new strands. Each DNA molecule now consists of one "old" strand and one "new strand. The two DNA molecules are identical to the one molecule with which you started.

F. Illustrate the replication of your DNA below.

## APPENDIX AII.

## Y CHROMOSOME

**Instructor's Guide:**

**Time Frame**: Part of 1 day field trip

**Target Group**: Advanced Placement Biology students

**Preparation of materials:**

1. Reagents:

- ✓ 0.1M citric acid (192.1 g/L)/5 = 3.84 g/ 200ml $dH_2O$
- ✓ 0.2M Na2HPO4 (141.96g/L)/5 = 5.68g/ 200ml $dH_2O$
- ✓ 50.4ml citric acid + 69.6ml $Na_2HPO^4$ =100ml buffer
- ✓ 3g Sucrose in 5ml of unused buffer
- ✓ 0.5% quinacrine dihydrochloride (0.5g/100ml $dH_2O$) or (0.3g/ 60ml $dH_2O$)
  - ☞ Note: solution is good 1-2wks if refrigerated and kept dark.
  - ☞ Caution: Quinacrines are carcinogenic. Use blotting paper and do not ingest.

2. Other Materials:

- ✓ Toothpicks
- ✓ Alcohol burner
- ✓ Microscope slides and coverslips
- ✓ Blotting paper
- ✓ Gloves
- ✓ Aluminum Foil
- ✓ Distilled water in water bottle
- ✓ Stopwatch

**Background Information for the teacher:**

🕮 Heterochromatin has been divided into two types. Constitutive heterochromatin is always condensed during interphase. Faculatative heterochromatin is composed of sequences that may be euchromatic in some developmental or physiological states, and heterochromatic in others, implying that genes may or may not be active

**Problems encountered:** lack of time

**References:**


1. Dan O'Mally room B123 cytogenetics lab in Life Science Bldg MSU. Phone 355-2731
2. MSU cytogenetics lab manual : qbands (QBAND)
3. http://www.udl.es/usuaris/e4650869/docencia/practiques_online/ cariotips/
4. http://www.hos.ufl.edu/mooreweb/AdvancedGenetics/10-21-99/10-21-99.html
5. Campbell, Neil, et al. 1999. Biology, Benjamin/Cummings, Melano Park, CA.

# Y CHROMOSOME

**Background:**
Quinacrine mustard solution is a fluorescent stain used to identify specific chromosomes and structural rearrangements. It is especially useful for distinguishing the Y chromosome from the other groups of chromosomes. The Y chromosome has the most heterochromatin, which is highly condensed DNA that is not transcribed. Quinacrine is a passive stain, which works its way into the chromosome and binds to specific histones (H1) responsible for DNA packaging. H1 binds to DNA adjacent to a nucleosome; the string of nucleosomes coil to form a chromatin fiber that is 30nm in diameter. The Y chromosome fluoresces the brightest because it contains the highest concentration of these histones.

**Materials:**
- ✓ Toothpicks
- ✓ Alcohol burner
- ✓ Microscope slides and coverslips
- ✓ Blotting paper
- ✓ Gloves
- ✓ Aluminum Foil
- ✓ Distilled water in water bottle
- ✓ Stopwatch

**Procedure**
1. Obtain a sample of cheek cells by lightly scraping the inside of your cheek with a toothpick.
2. Transfer the cheek cells to a microscope slide
3. Quickly run your slide and cheek cells through a low flame to dry and fix your sample.
4. Add 3 drops of quinacrine mustard stain to your slide and let it stand for 5-15 minutes.
5. Gently rinse the slide with a distilled water bottle for 30 seconds or until color disappears from $H_2O$.
6. Add 5 drops of buffer solution to specimen and let sit for 1 minute.
7. Lightly tap excess buffer from slide.
8. Add 2-3 drops of sucrose solution and mount the coverslip. Remove excess sucrose from the top of the coverslip with tissue.
9. View slide with fluorescence microscope with appropriate filters. (Note: 445 wavelength is recommended).
10. Observe and compare a cell from someone of the opposite sex.

## APPENDIX AIII.

## MANIPULATING DNA REPLICATION

**Instructor's Guide**

**Time frame:** 1 class period

**Target group:** Advanced Placement Biology students

**Background information for the teacher:** The abstract concepts in molecular biology are a challenge to concrete thinkers. The "real" pipe cleaners are used as a sensory tool to understand the abstract process of DNA replication. Each student is given an unassembled kit (see materials below) to construct as the teacher explains the process of DNA replication. Note: Kits for each student are very inexpensive and compact enough to take home for practice.

**Preparation of materials:**

Place the following materials in a ziploc bag
1-12" red pipecleaner
1-12" green pipecleaner
1-6" shiny red pipecleaner
2-3" shiney green pipecleaners
3-1" white pipecleaners
2- paper hole reinforcement labels
4-self adhesive file folder labels
1-purple pony bead
1-green pony bead
3-pink pony beads

**Sources of materials:** Michael's craft store
**Problems encountered:** showing how polymerase only reads 5'→ 3'
**References:** "Modeling DNA Replication" American Biology Teacher, Vol 60, No. 6, June 1998

## MANIPULATING DNA REPLICATION

**Objective:** To use pipe cleaners and beads to model the process of DNA replication.

**I. DNA Structure**

A. Two 12" long pipe cleaners are wound around each other to represent the DNA double helix.
- The red pipe cleaner represents one of the original DNA template strands.
- The green pipe cleaner represents the other parent DNA strand

B. The two strands of DNA are antiparallel (their sugar phosphate backbones run in opposite directions.
- Label one end of your red pipe cleaner as $5'PO_4^-$
- Label one end of your green pipe cleaner as 3'OH respectively

**II. DNA Separation and Unwinding**

A. A branch point in a replication bubble at which DNA synthesis occurs is called a replication fork.
- *Autoradiographic studies have shown that DNA synthesis almost always proceeds in both directions from the point where replication is initiated*
- *Figure 16.6 show picture of bidirectional replication).*

B. Helicase attaches to the replication fork and uses energy derived from hydrolysis of ATP to drive the unwinding and separation of the DNA molecule.
- Since 2 ATP molecules are required for unwinding each base pair, donut-shaped paper hole reinforcements labeled "ATP" are attached to the helicase bead before it is threaded along the length of the wound DNA strands.

1. The replication fork is produced by threading a purple "pony bead" 2/3 of the way down the double helix, unwinding the two strands, to simulate the action of the enzyme helicase.
   - DNA Gyrase *(grey bead)* relaxes supercoils created by helicase. (As unwinding continues, compensating DNA turns accumulate downstream of the replication fork.). DNA gyrase cuts both strands, allowing them to rotate and unwind, relieving the built-up tension. The ends are then resealed by the gyrase.

2. After DNA helicases unwind the DNA, the exposed single stranded segments are subject to nuclease digestion and breakage. To prevent this, single-stranded segments are immediately bound by single stranded binding protein (ssb), which hold the unpaired DNA strands apart to be replicated.).
   - DNA bound to SSB's is semirigid w/ no bends, which facilitates DNA synthesis.
   - **"Helicase hacks"** our double helix into two strands

- The DNA is now ready for both leading and lagging strand synthesis

## III. Leading Strand Synthesis

A. The new complementary strands are synthesised by polymerase III (pink bead)
   - **"Polymerase pastes"** nucleotides alongside the template

B. DNA polymerase has several limitations that contribute to the complexity of the replication process.

   1. Nearly all known DNA polymerases can add a nucleotide only to the free 3'-OH group of a base-paired polynucleotide so that DNA chains are extended only in the 5'→ 3' direction.

   2. DNA polymerases can add nucleotides only to the 3'end of an existing polynucletide strand.

C. Initiation of new chain growth is accomplished by synthesizing a short segment of RNA with a free 3'OH group. A 1" piece of white pipe cleaner (RNA primer) is wound around the far end of the single DNA strand which has the free 3'OH (not labeled).
   - The primase component of the primosome is responsible for synthesis of the short RNA fragment. Both the leading and lagging strands require an RNA primer provided by the primase.

D. On the end of the white RNA primer that faces the replication fork, place a pink pony bead (DNA polymerase III).

E. A 6" shiny red pipe cleaner (leading strand of DNA) is attached to the DNA polymerase. The leading strand is wound around that same single DNA strand starting at the white RNA primer and heads into the replication fork
- The two parent strands are replicated in different ways.

F. The newly synthesized DNA strand that extends 5'→ 3' in the direction of the replication fork movement, the leading strand, is continuously synthesized. Only one priming event is required to initiate synthesis of the leading strand.

## IV. Lagging Strand Synthesis

A. The other new strand, the lagging strand, is also synthesized in the 5'→3" direction. However, this new strand, called an Okazaki fragment, can only be made discontinuously.
   - Each Okazaki fragment is only 100-200 nt long and requires multiple priming sites due to the 5' → 3' synthesis requirement of DNA polymerase.

B. A second 1' white RNA primer, which serves to initiate Okasaki fragment synthesis, is wrapped around the other single DNA strand at the replication fork.
   - Remember, on the leading strand the white RNA primer was at the opposite end of the single stranded DNA.

C. Next, a 2" shiny green pipe cleaner (Okasaki fragment) is attached to a second pink bead (DNA polymerase III). Both are wound around the DNA template, heading in the direction out of the fork.

D. Beyond that shiny green segment, another white RNA primer is wound, and further "out" an adjacent shiny Green Okasaki fragment and pink polymerase II is added.
   - The RNA primer between each Okazaki fragment is removed by DNA polymerase I. (the RNA segment is degraded to free nucleotides) Then, deoxynucleotides fill in the gap and are polymerized by DNA polymerase III.

E. DNA ligase (lime bead) is symbolically attached to the starting end of the Odasaki fragment. Ligase catalyzes the formation of a phosphoester bond between the 5'-phosphate and 3'hydroxyl to yield a continuous strand of complementary DNA
- "**Ligase links**" the Okazaki fragments (lagging DNA)

# MANIPULATING DNA REPLICATION

**Objective:** To use pipe cleaners and beads to model the process of DNA replication.

## I. DNA Structure

A. Two 12" long pipe cleaners are wound around each other to represent the _____.

B. The two strands of DNA are _____ (_____ _____).

## II. DNA Separation and Unwinding

A. A branch point in a replication bubble at which DNA synthesis occurs is called a _____.

B. _____ attaches to the replication fork and uses energy derived from hydrolysis of ATP to drive the unwinding and separation of the DNA molecule.

1. The replication fork is produced by threading a purple pony bead (_____) 2/3 of the way down the double helix and unwinding the two strands.

2. After DNA helicases unwind the DNA, the exposed single stranded segments are subject to nuclease digestion and breakage. To prevent this, single-stranded segments are immediately bound by _____ _____, which hold the unpaired DNA strands apart to be replicated.).

- _____

## III. Leading Strand Synthesis

A. The new complementary strands are synthesised by _____

- _____

B. DNA polymerase has several limitations that contribute to the complexity of the replication process.

1. Nearly all known DNA polymerases can add a nucleotide only to the free 3'-OH group of a base-paired polynucleotide so _____ _____.

2. DNA polymerases can add nucleotides only to the _____ _____.

C. Initiation of new chain growth is accomplished by synthesizing a short segment of RNA with a free 3'OH group. A 1" piece of white pipe cleaner (_____ ) is wound around the far end of the single DNA strand which has the 3"OH label.

D. On the end of the white RNA primer that faces the replication fork, place a pink pony bead (_____).

E. A 6" shiny red pipe cleaner (_____) is attached to the DNA polymerase. The leading strand is wound around that same single DNA strand starting at the white RNA primer and heads into the replication fork

F. The newly synthesized DNA strand that extends 5'→ 3' in the direction of the replication fork movement, called the _____, is _____ synthesized.

## IV. Lagging Strand Synthesis

A. The other new strand, _____, is also synthesized in the 5'→3" direction. However, this new strand, called an _____, can only be made _____.

B. A second 1' white RNA primer, which serves to initiate Okasaki fragment synthesis, is wrapped around the other single DNA strand at the replication fork.

C. Next, a 2" shiny green pipe cleaner (_____) is attached to a second pink bead (_____). Both are wound around the DNA template, heading in the direction out of the fork.

D. Beyond that shiny green segment, another white RNA primer is wound, and further "out" an adjacent shiny geen Okasaki fragment and pink polymerase II is added.

E. _____ (lime bead) is symbolically attached to the starting end of the Okasaki fragment. Ligase catalyzes the formation of a phosphoester bond between the 5'-phosphate and 3'hydroxyl to yield a continuous strand of complementary DNA.

_____

## APPENDIX AIV.

## BACTERIAL TRANSFORMATION PRE-LABORATORY

**Instructor's Guide:**

### I. Key Concepts 1: Bacterial Transformation

Define transformation.

- A phenomenon in which external DNA is assimilated by a cell.

What gene are you introducing?

- a gene for resistance to the antibiotic ampicillin is introduced into a bacterial strain that is killed by ampicillin.

How do you know it was taken up?

- If the susceptible bacteria incorporate the foreign DNA, they will become ampicillin resistant.

### II. Bacterial Colonies

What are some of the materials required in this lab?

- Escherichia coli, Luria Broth agar and petridishes

How many individual cells are in each colony (roughly)?

- Each colony in the petri dish is made up of millions of individual cells.

### III. *E. coli* Bacteria

Name one place *E. coli* is found?

- the human gut

Describe two places *E.coli* stores it's genetic material?

- It has genetic material in the bacterial chromosome and some E. coli store genetic material in plasmids

### IV. Plasmids

Define plasmids

- A small ring of DNA that carries accessory genes separate from those of a bacterial chromosome. Also found in some eukaryotes, such as yeast.

What are plasmids used for in genetic engineering?

- In genetic engineering, plasmids are one means used to introduce foreign genes into a bacterial cell.

Differentiate between an +amp R cell and -amp R cell
- Some plasmids have the ampR gene, which confers resistance to the antibiotic ampicillin. E. coli cells containing this plasmid termed "+ampR" cells, can survive and form colonies on LB agar that has been supplemented with ampicillin. In contrast, cells lacking the ampR plasmid, termed "–ampR" cells, are sensitive to the antibiotic, which kills them.

## V. Competent Cells

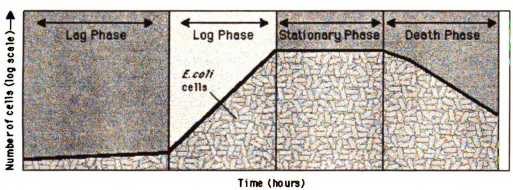
Define a competent cell.
- Competent cells are more likely to incorporate foreign DNA because their cell walls are altered so that DNA can pass through more easily.

How do you make a competent cell?
- Cells are made competent by a process that uses calcium chloride and heat shock.

When is it easier to make a cell competent?
- Cells that are undergoing very rapid growth are made competent more easily.

Explain why each phase has it's particular # of cells?
- The growth rate of a bacterial culture is not constant. In the early hours (lag phase), growth is very slow because the starting number of dividing cells is small. This is followed by a time rapid cell division known as the log phase.

Draw & label the 4 phases of cell division




## VI. Design of the Experiment

List 5 techniques of sterile procedure.
- Always wash your hands and work surface before beginning.
- Always keep the lid of the petri dish on it or over it at all times.
- Never touch the end of a tool that touches bacteria.
- Always keep hair pulled back and use goggles when flame is present.
- Always wash your hands thoroughly with soap and hot water before leaving the lab.

## VII. Transformation Procedure

Why do you also prepare a second group of *E. coli* cells?

- to verify that E. coli will not grow on agar with ampicillin unless it is transformed, and that nothing in the procedure itself affects the survival of E. coli.

What is the only difference in your control group?

- The control group lacks the ampR plasmids

Describe the 6 Steps in this procedure

- Ampicillin sensitive *E. coli* cells are transferred to cold CaCl2.
- The $amp^R$ plasmids are added only to the experimental cells.
- Both cells are heat shocked at 42°C.
- The treated cells are spread on LB plates containing ampicillin
- All plates are incubated for 24 hours
- Count *E.coli* colonies and determine if cells have been transformed.

## BACTERIAL TRANSFORMATION PRE-LABORATORY

### I. Key Concepts 1: Bacterial Transformation

Define transformation.

_____
_____

What gene are you introducing?

_____
_____

How do you know it was taken up?

_____
_____

### II. Bacterial Colonies

What are the materials required in this lab?

_____
_____

How many individual cells are in each colony (roughly)?

_____
_____

### III. *E. coli* Bacteria

Name one place *E. coli* is found?

_____
_____

Describe two places *E.coli* stores it's genetic material?

_____
_____

### IV. Plasmids

Define plasmids

_____
_____

What are plasmids used for in genetic engineering?

_____
_____

Differentiate between an +amp R cell and -amp R cell

## V. Competent Cells

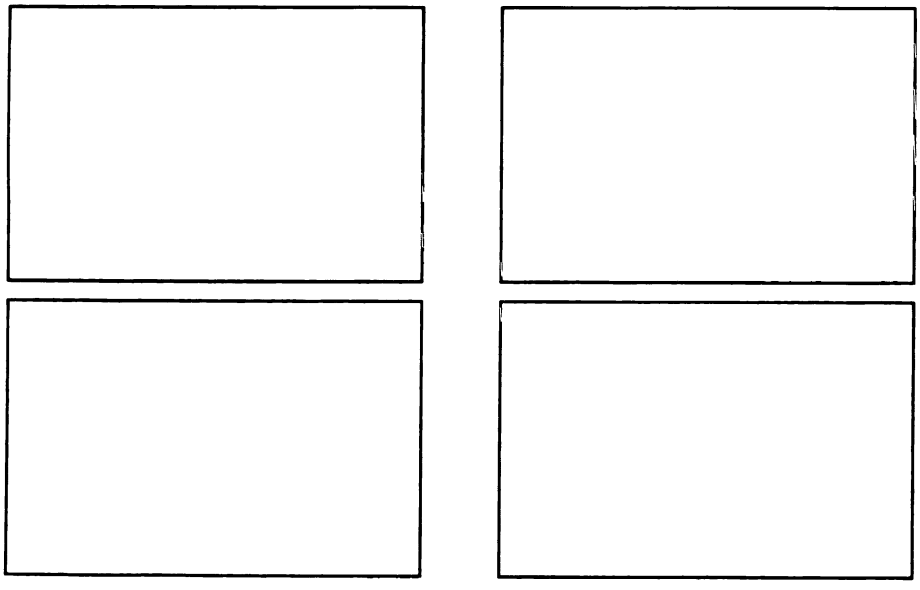
Define a competent cell.

How do you make a competent cell?

When is it easier to make a cell competent?

Explain why each phase has it's particular # of cells?

Draw & label the 4 phases of cell division



## VI. Design of the Experiment

List 5 techniques of sterile procedure.

## VII. Transformation Procedure

Why do you also prepare a second group of *E. coli* cells?

What is the only difference in your control group?

## VIII. Describe the 6 Steps in this procedure

## APPENDIX AV.

## BACTERIAL TRANSFORMATION LABORATORY

**Instructor's Guide:**

**Time Frame:** 2 hours

**Target Group:** Advanced Placement Biology students

**Preparation of materials:** see Carolina Biological Teacher Guide

**Sources of materials:** Carolina Biological Supply Company (catalog # 21-1142)

**Problems encountered:** Students' water temperatures were not exact. I plan on using a water bath instead of beakers of water on hot plates.

**Reference:** Carolina Biological Colony Transformation Kit
AP Biology Laboratory Manual for Teachers and Students
Lab 18: Molecular Genetics: Recombinant DNA

**Answers to Questions in the Student Laboratory:**

**RESULTS**

1. Observe the bacterial growth through the bottom of the culture plates. **Do not open the plates**. Count the number of individual colonies (use a permanent marker to mark off each colony as it is counted). If cell growth is too dense to count individual colonies, record "lawn." Share results on Positive Controls with another lab group.

| | | | |
|---|---|---|---|
| Positive Control: (LB+) | ✎ lawn | Positive Control: (LB-) | ✎ lawn |
| Experimental: (LB/Amp+) | ✎ 5-500 | Negative Control: (LB/Amp-) | ✎ 0 |

**INTERPRETATION AND CONCLUSIONS**

1. Compare and contrast the number of colonies on each of the following pairs of plates. What does each pair of results tell you about the experiment?

a) LB+ and LB- : ✎ Cells are viable. In the absence of antibiotics both cells grow equally well.

b) LB/Amp- and LB/Amp+ : ✎ Only transformed cells grow in the presence of ampicillin.

c) LB/Amp+ and LB+ : ✎ Transformation is a rare event, only 1 in 1million

cells is transformed.

d) LB/Amp- and LB- : ✎ Wild-type cells fail to grow in the presence of ampicillin. The antibiotic is active.

2. Transformation efficiency is expressed as the number of antibiotic resistant colonies per μg of pAMP DNA. Because transformation is limited to only those cells that are competent, increasing the amount of plasmid used does not necessarily increase the probability that a cell will be transformed. A sample of competent cells is usually saturated with small amounts of plasmid and excess DNA may actually interfere with the transformation process. The object is to determine the mass of pAMP that was spread on the experimental plate and was, therefore, responsible for the transformants observed.

   a) Determine total mass (in μg) of pAMP used in Step 8.
   [Mass = Concentration x Volume].
   ✎ 0.005μg/μL * 10μL = 0.05μg

   b) Calculate the total volume of cell suspension prepared. [Steps 2 and 13]
   ✎ 250μL + 250μL = 500μL

   c) Calculate the fraction of the total cell suspension that was spread on the plate. [Volume Suspension Spread/Total Volume Suspension (part b)= Fraction Spread]
   ✎ 100μL / 500μL = 0.2

   d) Determine the mass of pAMP in cell suspension. [Total mass of pAMP (part a) X fraction spread (part b) = Mass pAMP Spread].
   ✎ 0.05μg * 0.2 = 0.01μg

   e) Determine number of colonies per μg pAMP. Express answer in scientific notation. [Colonies Observed/Mass pAMP Spread (part c) = Transformation Efficiency].
   ✎ Ex: ("100 colonies" / 0.01μg) = 1x $10^4$ transformants/μg

3. What factors might influence transformation efficiency? Explain the effect of each you mention.
   ✎ Technique errors (such as not taking large enough cell masses, not resuspending the cells, missing or indistinct heat shock). These could decrease or even eliminate colony growth on plates
   ✎ Growth stage of cells transferred could effect the transformation efficiency because healthy, rapidly dividing cells are more likely to uptake DNA.
   ✎ As the cell solution becomes saturated with DNA, additional plasmid DNA could decrease the transformation efficiency.

# BACTERIAL TRANSFORMATION LABORATORY

## INTRODUCTION

### Background Information

The bacterium Escherichia coli (E. coli) is an ideal organism for genetic manipulation and has been used extensively in recombinant DNA research. It is a common inhabitant of the human colon and can easily be grown in standard nutrient mediums.

The single circular chromosome of E. coli contains 5 million DNA base pairs (1 /600th the total amount of DNA in a human cell). In addition, the cell contains small, circular; extrachromosomal (outside the chromosome) DNA molecules called plasmids. These fragments of DNA, 1,000 to 200,000 base pairs in length, also carry genetic information. Some plasmids replicate only when the bacterial chromosome replicates and usually exist only as single copies within the bacterial cell. Others replicate autonomously and often occur in as many as 10 to 200 copies within a single bacterial cell. Certain plasmids, called R plasmids, carry genes for resistance to antibiotics such as ampicillin, kanamycin, or tetracycline.

In nature, genes can be transferred between bacteria in three ways: conjugation, transduction, or transformation. Conjugation is a mating process during which genetic material is transferred from one bacterium to another "sexually" different type. Transduction requires the presence of a virus to act as a vector (carrier) to transfer small pieces of DNA from one bacterium to another. Bacterial transformation involves transfer of genetic information into a cell by direct absorption of the DNA released from a donor cell.

Through the process of bacterial transformation, a bacterium can acquire a new trait by incorporating and expressing foreign DNA. In the laboratory, the DNA used most commonly for transformation experiments is bacterial plasmid DNA. These plasmids often carry a gene for antibiotic resistance. The presence of the antibiotic-resistance gene makes it possible to select bacteria containing the plasmid of interest; the bacteria that contain the plasmid will grow on a medium that contains the antibiotic, whereas bacteria lacking the plasmid will not be resistant to the antibiotic and will die. Transformation can occur naturally, but the incidence is extremely low and is limited to a relatively few bacterial strains. During the growth cycle of these strains, there exists a short period of time when the bacteria are most receptive to uptake of foreign DNA. At this stage the cells are said to be competent (Competence to absorb DNA usually develops toward the end of the logarithmic growth phase, just before cells enter the stationary phase in culture.) The mechanism by which competence is acquired is not completely understood, but in the laboratory, treating bacterial cells with divalent cations such as $Ca^{2+}$ and $Mg^{2+}$ can induce the competent state.

In this exercise, you will simulate the construction of a recombinant plasmid. Plasmids can transfer genes such as those for antibiotic resistance which are already a part of the plasmid, or plasmids can act as carriers for introducing foreign DNA from other bacteria, plasmids, or even eukaryotes into bacterial cells. Restriction

endonucleases are used to cut and insert pieces of foreign DNA into the plasmid vectors (Figure 1).

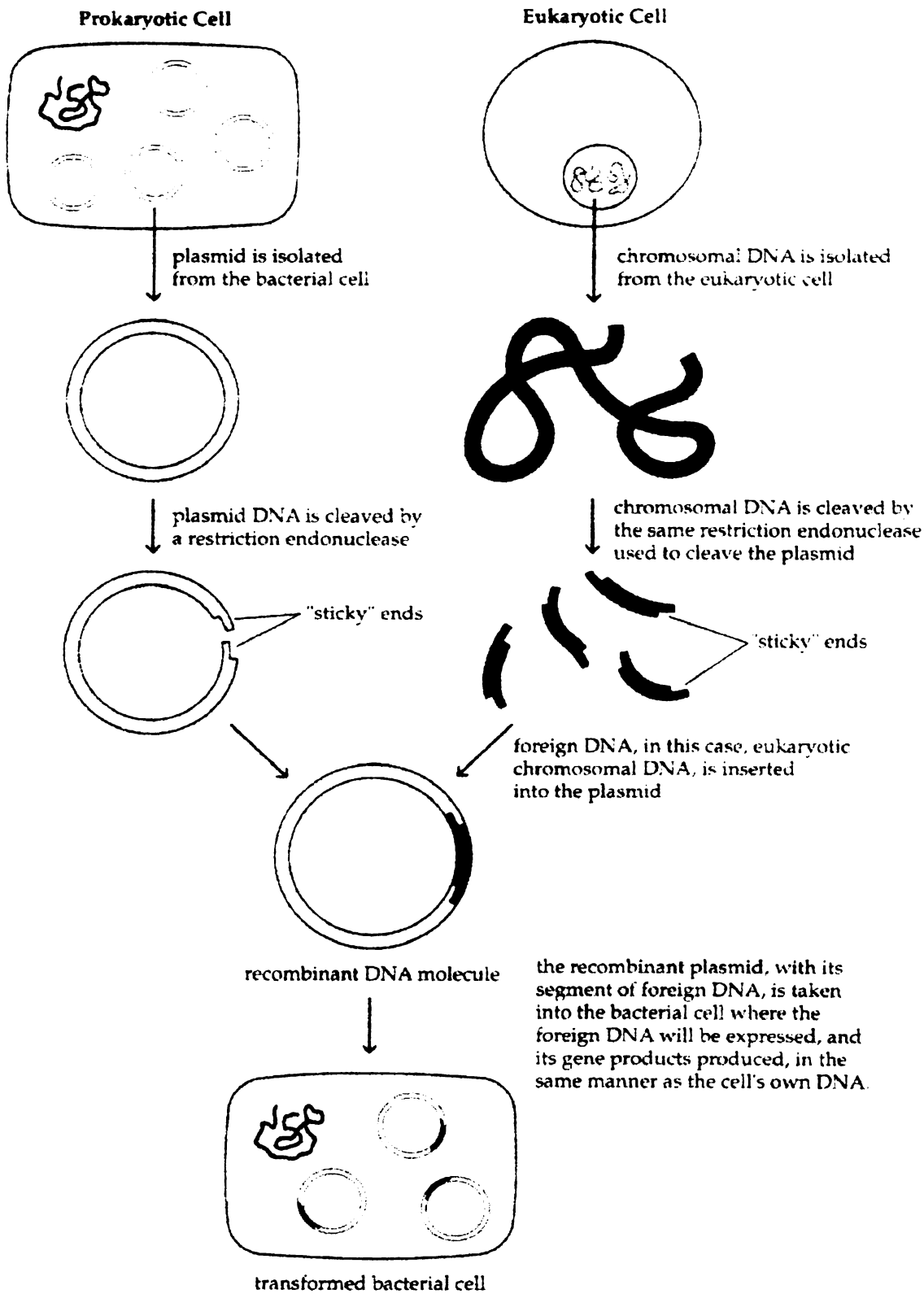



**Figure 1:** A typical recombinant DNA experiment using a restriction endonuclease.

Each restriction endonuclease "recognizes: a specific DNA sequence (usually a 4 to 6 base-pair sequence of nucltotides in double stranded DNA and digests phosphodiester bonds at specific sites in the sequence (recall that phosphodiester bonds link one nucleotide to the next in a DNA polynucleotide chain). If circular DNA is cut at only one site, an open circle results. If the restriction endonuclease recognizes two or more sites on the DNA molecule, two or more fragments will result. The length of each DNA fragment corresponds to the distance between restriction sites (restriction sites flank the fragment at its ends). Some restriction endonucleases cut cleanly through the DNA helix at the same position on both strands to produce fragments with blunt ends. Other endonucleases cut specific nucleotides on each strand to produce fragments with overhangs or "sticky ends" (Figure 2). Using the same restriction endonuclease to cut DNA from two different organisms produces complementary sticky ends, which can be realigned in a "template-complement" manner, thus recombining the DNA from the two sources (Figure 2).

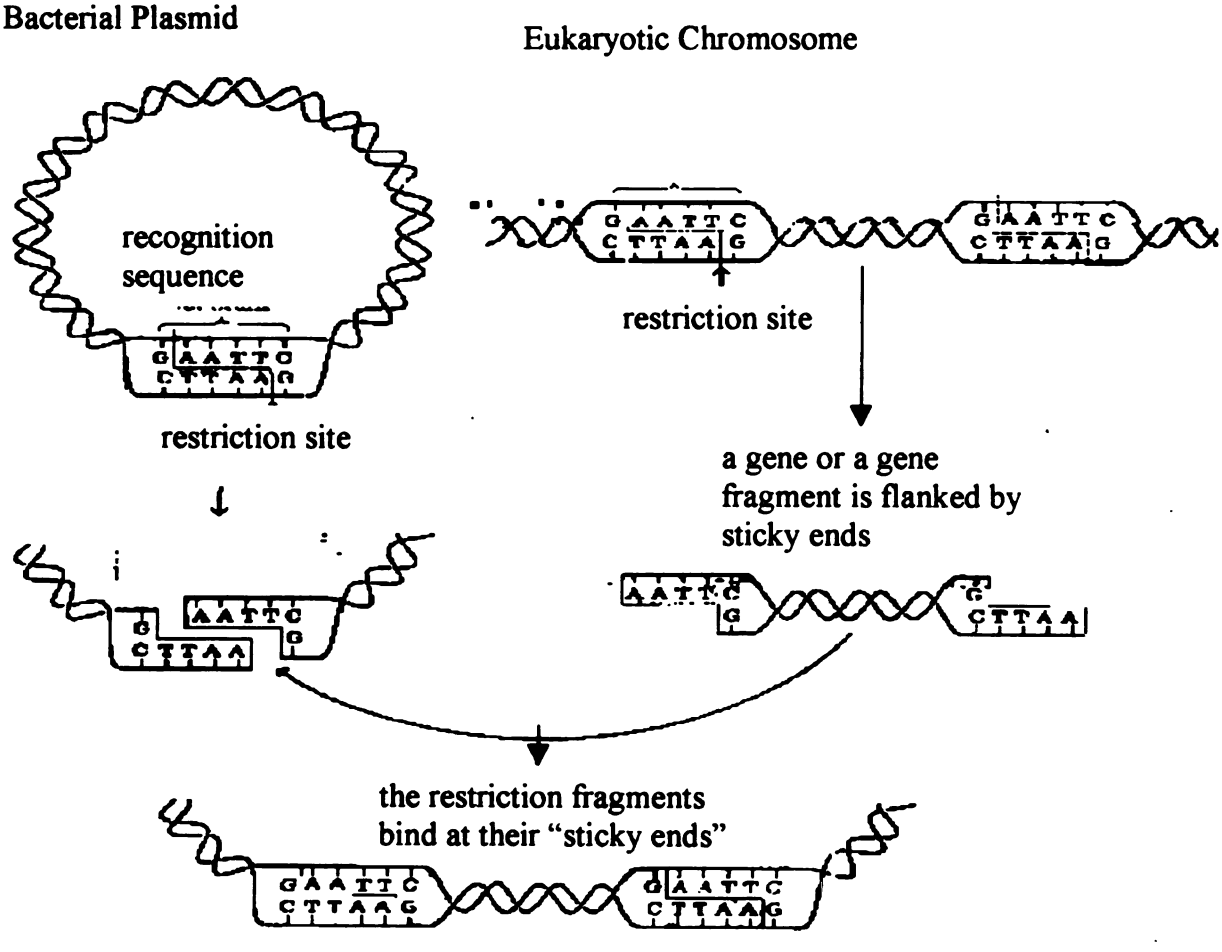



**Figure 2** EcoRI endonuclease cleaving a bacterial plasmid and a fragment of eukaryotic DNA and then recombining them.

In bacteria, restriction enzymes provide protection by breaking and destroying the DNA of invaders, such as that of bacteriophage viruses. However, since the recognition sites for restriction endonucleases also occur within the bacterial DNA itself, bacteria have a mechanism for preventing their own restriction enzymes from digesting their own DNA. For each restriction endonuclease produced by a bacterium, there is a corresponding enzyme that methylates the bacterial DNA at that enzyme's specific recognition site: addition of a methyl group to the nucleic acid base prevents a close association from forming between the restriction endonuclease and the recognition site. In this way, bacteria can break down the DNA of invaders while protecting their own genetic material from destruction.

**Objectives**

- To understand the principles of bacterial transformation
- To understand the conditions under which cells can be transformed
- To understand the process of competent cell preparation
- To understand how a plasmid can be engineered to include a piece of foreign DNA
- To understand how plasmid vectors are used to transfer genes
- To understand how restriction endonucleases function
- To understand how antibiotic resistance is transferred between cells
- To use sterile technique
- To calculate transformation efficiency

**MATERIALS: setup for each group (four Ss per group)**

- ✓ E. coli culture-starter plate
- ✓ 1- marker
- ✓ 2- 15ml tubes
- ✓ p-AMP plasmid
- ✓ 4- 1ml sterile transfer pipette
- ✓ 500uL- 0.05-M $CaCl_2$ ice cold
- ✓ Bunsen burner
- ✓ inoculating loop
- ✓ spreading rod
- ✓ water-bath 42° C
- ✓ 3ml Luria broth
- ✓ 2 Luria broth agar plates
- ✓ 2- LB/ ampicillin agar plates
- ✓ 1- 37°C incubator

## METHOD/ PROCEDURE

**Step by step**

1. Mark one sterile 15-mL tube "+AMP "; this tube will have the plasmid added to it. Mark another tube "-AMP "; this tube will not have the plasmid added.

2. Use a sterile micropipette (Fig. 1) to add 250 microliters (μL) of ice cold *0.05M* $CaCl_2$ to each tube.

3. Place both tubes on ice.

4. Use a sterile inoculating loop to transfer one or two large (3mm) colonies of *E. coli* cells from a starter plate to "+AMP" tube using a sterile inoculating loop.
   a) Try to get the same amount of bacteria into each tube.
   b) Be careful not to transfer any agar from the plate along with the cell mass.
   c) Vigorously tap the loop against the wall of the tube to dislodge the cell mass.
   d) Hold the tube up to light to observe cell mass fall off of the loop.

5. Immediately mix the suspension by repeatedly drawing in and emptying out a sterile micropipette with the suspension.

6. Return "+AMP" Tube to ice. Transfer second mass of cells to "-AMP " Tube, and suspend as described in Step 3.

7. Return "-AMP " Tube to ice. Both tubes should now be on ice.

8. Use a sterile inoculating loop to add one loopful of pAMP solution (0.005 [μg/μL) directly into the cell suspension in tube "+AMP".
   a) Note 10 μL is measured when DNA solution forms a "bubble" across the loop opening.
   b) Swish the loop to mix the DNA. Then mix by tapping the tube with your finger.
   c) This solution contains the antibiotic resistance plasmid.

9. Return "+AMP" Tube to ice, and incubate both tubes on ice for 15 minutes.

10. While the tubes are on ice, label each agar plate (1LB and 2 LB/Amp) on the bottom with lab group name and date.
    a) Label one LB/Amp plate "LB/Amp +" This is the experimental plate.
    b) Label the other LB/Amp plate "LB/Amp -" This is the negative control.
    c) Label one LB plate either "LB+" or "LB-" as directed by your instructor. This is a positive control

11. A brief pulse of heat facilitates entry of foreign DNA into the *E. coli* cells. Following the 15 minute incubation, heat-shock cells in both the "+AMP" and "-AMP" tubes.
    a) Carry the beaker containing ice and tubes to the water bath.
    b) Remove both tubes directly from the ice and immediately immerse them in the 42° C water bath for 90 seconds.

12. Immediately return cells to ice for two or more minutes.

13. Use a sterile micropipette to add 250μL of Luria broth to each tube.
    a) Mix by gently tapping with your finger

b) Set the tubes in a test tube rack at room temperature.
c) Any transformed cells are now resistant to ampicillin because they possess the gene whose product renders the antibiotic ineffective.

14. Use a sterile micropipet to add 100μL cell suspension from "-AMP" Tube onto "LB/Amp-" Plate. If your group has been assigned the -AMP Positive Control, add another 100μ L onto "LB-" Plate.

15. Immediately spread cells over the surface of plate(s) using a sterile-spreading rod.
    a) Dip cell spreader in ethanol, and briefly pass it through Bunsen flame to ignite alcohol before spreading each plate. Allow the alcohol to burn off away from Bunsen flame and alcohol container.
    b) Lift the plate lid only enough to insert spreader and cool spreader by touching it to the side of the agar away from cell suspension.
    c) Use the spreader to evenly distribute cell suspension over the surface of the plate, and replace the plate lid.
    d) Return the cell spreader to ethanol without flaming.

16. Use the sterile micropipet to add 100μL cell suspension from +AMP Tube onto +Amp Plate. If your group has been assigned the +AMP Positive Control, add another 100μL onto +LB Plate.

17. Immediately spread cell suspensions as described in Step 15.

18. Allow the plates to set for several minutes. Then wrap them together with tape. Place the plates upside down in 37° C incubator, and incubate for 12 to 24 hours.

## RESULTS

1. Observe the bacterial growth through the bottom of the culture plates. **Do not open the plates**. Count the number of individual colonies (use a permanent marker to mark off each colony as it is counted). If cell growth is too dense to count individual colonies, record "lawn." Share results on Positive Controls with another lab group.

   Positive Control: (LB+) _____

   Positive Control: (LB-) _____

   Experimental: (LB/Amp+) _____

   Negative Control: (LB/Amp-) _____

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## INTERPRETATION AND CONCLUSIONS

**Questions**

1. Were results as expected? If not, explain possible reasons for variations from expected results.

   _____

   _____

2. Compare and contrast the number of colonies on each of the following pairs of plates. What does each pair of results tell you about the experiment?
   a) LB+ and LB- :

   _____

   b) LB/Amp- and LB/Amp+ :

   _____

   c) LB/Amp+ and LB+ :

   _____

   d) LB/Amp- and LB- :

   _____

3. Transformation efficiency is expressed as the number of antibiotic resistant colonies per μg of pAMP DNA. Because transformation is limited to only those cells that are competent, increasing the amount of plasmid used does not necessarily increase the probability that a cell will be transformed. A sample of competent cells is usually saturated with small amounts of plasmid and excess DNA may actually interfere with the transformation process. The object is to determine the mass of pAMP that was spread on the experimental plate and was, therefore, responsible for the transformants observed.

   a) Determine total mass (in μg) of pAMP used in Step 8.
      [Mass = Concentration x Volume].

      _____

   b) Calculate the total volume of cell suspension prepared. [Steps 2 and 13]

      _____

   c) Calculate the fraction of the total cell suspension that was spread on the plate.
      [Volume Suspension Spread/Total Volume Suspension (part b)= Fraction Spread]

      _____

   d) Determine the mass of pAMP in cell suspension. [Total mass of pAMP (part a) X fraction spread (part b) = Mass pAMP Spread].

      _____

e) Determine number of colonies per μg PAMP. Express answer in scientific notation. [Colonies Observed/Mass pAMP Spread (part c) = Transformation Efficiency].

4. What factors might influence transformation efficiency? Explain the effect of each you mention.

## APPENDIX AVI.

## GEL ELECTROPHRESIS PRE-LABORATORY

**Instructor's Guide:**

### I. Key Concepts

What types of organisms have restriction enzymes?

- bacteria

Define restriction enzymes.

- A degradative enzyme that recognizes and cuts up DNA (including that of certain phages) that is foreign to a bacterium.

Define recognition sequence.

- A specific sequence of nucleotides at which a restriction enzyme cleaves a DNA molecule.

### II. How do restriction enzymes work?

Why use restriction enzymes?

- They cut DNA only within very precise recognition sequences.

Recognition sites are palendromic, what does that mean?

- This means that the recognition sequence on one DNA strand reads in the opposite direction on the complementary strand.

### III. Cutting DNA with Restriction Enzymes

What does the prefix "micro" mean?

- the prefix "micro-" indicates one-millionth. It is symbolized by μ,

### IV. Gel Electrophoresis

What is gel electrophoresis?

- a procedure that separates molecules on the basis of their rate of movement through a gel under the influence of an electrical field.

In gel electrophoresis, what is the direction of movement affected by?

- the charge of the molecules

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In gel electrophoresis, what affects the rate of migration?

- the rate of movement is affected by their size and shape, the density of the gel, and the strength of the electrical field.

What is the charge of DNA?

- DNA is a negatively charged molecule

When DNA has been cut by restriction enzymes, what fragments will migrate the farthest?

- Because the smallest fragments move the most quickly, they will migrate the farthest during the time the current is on.

## V. Design of the Experiment

Where are the 3 samples of DNA taken from

- a virus, the bacteriophage lambda

What are the 3 samples of DNA being used

- One sample will be uncut DNA, one will be incubated with the restriction enzyme HindIII, and one will be incubated with EcoRI.

## VI. Preparing the Gel

List the 4 main steps in preparing the gel

- Mix agarose & buffer
- boil mixture in microwave
- cool mixture to 65°C and pour into gel tray
- let gel solidify at room temperature

What end are the wells placed in the electrophoresis chamber?

- the wells are placed at the negative electrode end

## VII. Loading the Gel

What two mixtures are added to the DNA and why?

- tracking dye- the molecules move at a faster rate than that of the DNA so you can see when to shut off the apparatus.
- sucrose or glycerol to make the mixture dense enough to sink into the well.

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## VIII. Filling the Wells

What do you want to avoid doing while filling the wells?

- do not puncture the bottom of the well with the micropipette!

## IX. Electrophoresis

When you turn on the power, what should move toward the positive electrode?

- the DNA / tracking dye

## X. Running the Gel

When do you turn off the power?

- When the tracking dye has moved near the end of the gel.

Staining and Photographing the DNA

What do you have to do to see the DNA

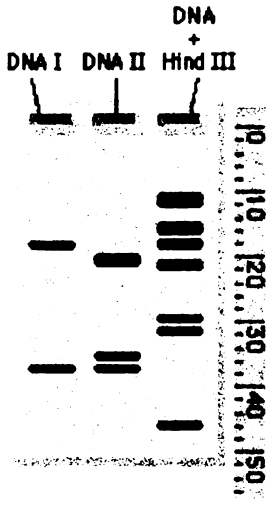
- add methylene blue, which will bind to the DNA and then rinse the gel repeatedly with water, so that the dye washes off the gel.

## XI. Analysis of Results II

How do researchers determine the size of DNA fragments produced with a particular restriction enzyme?

- they run the unknown DNA alongside DNA with known fragment sizes. The known DNA acts as a marker.

Complete the following table




| Actual Base Pairs (bp) | Measured Distance (mm) |
|---|---|
| 23,130 | 11 |
| 9416 | 16 |
| 6557 | 18 |
| 4361 | 22 |
| 2322 | 29 |
| 2207 | 32 |
| 564 | 46 |

Read the number of base pairs in the unknown fragment from the graph. Record your answers in the table below.

| | **Distance Migrated (mm)** | **Interpolated Fragment Size** (in base pairs) |
|---|---|---|
| **Fragment 1** | 20 | 4796 |
| **Fragment 2** | 34 | 1405 |
| **Fragment 3** | 36 | 1268 |

## GEL ELECTROPHRESIS PRE-LABORATORY

### I. Key Concepts

What type of organisms have restriction enzymes?

_____

Define restriction enzymes.

_____

_____

Define recognition sequence.

_____

_____

### II. How do restriction enzymes work?

Why use restriction enzymes?

_____

Recognition sites are palendromic, what does that mean?

_____

_____

### III. Cutting DNA with Restriction Enzymes

What does the prefix "micro" mean?

_____

### IV. Gel Electrophoresis

What is gel electrophoresis?

_____

_____

In gel electrophoresis, what is the direction of movement affected by?

_____

In gel electrophoresis, what affects the rate of migration?

What is the charge of DNA?

When DNA has been cut by restriction enzymes, what fragments will migrate the farthest?

## V. Design of the Experiment

Where are the 3 samples of DNA taken from

What are the 3 samples of DNA being used

## VI. Preparing the Gel

List the 4 main steps in preparing the gel

What end are the wells placed in the electrophoresis chamber?

## VII. Loading the Gel

What two mixtures are added to the DNA and why?

## VIII. Filling the Wells

What do you want to avoid doing while filling the wells?

---

## IX. Electrophoresis

When you turn on the power, what should move toward the positive electrode?

---

## X. Running the Gel

When do you turn off the power?

---

Staining and Photographing the DNA
What do you have to do to see the DNA

---

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## XI. Analysis of Results II

How do researchers determine the size of DNA fragments produced with a particular restriction enzyme?

---

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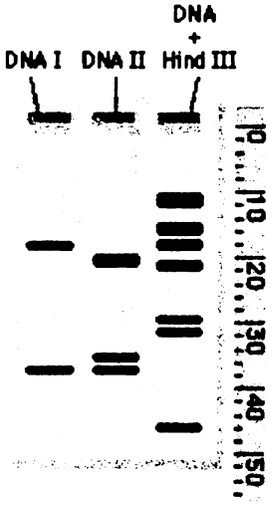
Complete the following table




| Actual Base Pairs (bp) | Measured Distance (mm) |
|---|---|
| 23,130 | |
| 9416 | |
| 6557 | |
| 4361 | |
| 2322 | |
| 2207 | |
| 564 | |

Read the number of base pairs in the unknown fragment from the graph. Record your answers in the table below.

| | **Distance Migrated (mm)** | **Interpolated Fragment Size** (in base pairs) |
|---|---|---|
| **Fragment 1** | | |
| **Fragment 2** | | |
| **Fragment 3** | | |

## APPENDIX AVII.

## GEL ELECTROPHORESIS OF λ DNA LABORATORY

**Instructor's Guide:**

**Time Frame:** 1 day field trip + 1 class period

**Target Group:** Advanced Placement Biology Students

**Preparation of materials:** The classroom set includes materials for 5 groups of students to perform the lab experiment.

1. Supplied ready to use:
   - ✓ Instant Lambda DNA (long white tubes)
   - ✓ Instant Restriction Enzyme EcoRl (red tubes)
   - ✓ Instant Restriction Enzyme HindIII (green tubes)
   - ✓ Instant Restriction Enzyme BamH1 (blue tubes)
   - ✓ Empty Microtubes (colored yellow)
   - ✓ Electrophoresis Box Gel Comb
   - ✓ Microsyringe Tips & Microsyringes
   - ✓ Bromophenol Blue Loading Dye (screw-topped tubes)
   - ✓ Foam Tray, for Holding Microtubes
   - ✓ Wires with Alligator Clips
   - ✓ 0.8% agarose gel, made by dissolving the agarose powder in diluted electrophoresis buffer: 1.2g of agarose in 150ml 1x TbE solution will give approximately 15 gels (each gel box holds about 10 mL agarose).

     Note: Do not dissolve the agarose in water. The easiest way to dissolve the agarose is to use a microwave oven. Heat to boiling to dissolve agarose. Stir frequently. Cool to 60 deg C before pouring if solution is prepared immediately before use. **Caution:** loosen all caps on containers when heating in a microwave.

2. Supplied, but requiring preparation:
   - ✓ Carbon Fiber Electrode Tissue, cut to approximately 42 x 22 mm

     Note: The carbon fiber material may release small fibers, which can cause minor skin irritations. It is a wise precaution to wear protective gloves.
   - ✓ Electrophoresis (TBE) Buffer Solution, prepared from the 20x concentrate supplied in the kit; add 25mL of 20x TBE to 475mL distilled or deionized water

     Note: the buffer solution may be saved and reused several times.

✓ Carolina BLU tm concentration DNA stain

3. Materials needed, but not provided:
   - ✓ 9V Batteries, 3 per gel
   - ✓ Distilled water to rehydrate the lambda DNA
   - ✓ Water bath set at 37°C, so that the DNA may be incubated with the restriction enzymes
   - ✓ Marking pens, to label the wells

**Background information for the teacher**

1. Casting agarose gels:
   a. Ensure that the comb teeth are set completely down on their "shoulders" when placed on the gel box, and covered to the precise depth with the agarose gel. Too shallow a well will result in some DNA splashing out due to vigorous unloading of the digital micropipette by students with unsteady hands. Wells should be able to accommodate 15 to 50 μL samples. Too deep a well may have a "shouldered connection" to the next well, allowing mixing with DNA cut by other restriction enzymes - or the controls - and the resulting bands will be blurred and indistinct.
   b. Pour the gels on unmovable counters, because it is inevitable that some students will bump tables while the gel is setting.
   c. Removing the comb after gel solidification must also be done very gently. Slowly lifting one end at a time reduces the number of ripped gel beds. (Note: Adding the running buffer before comb removal also helps.)

2. Loading gel beds:
   a. Be sure that students have identified their well sequence as starting from a particular side and have recorded this information in their lab handout, as well as what sample they have put into each well. Remind them to change pipette tips after loading each well.
   b. Using two hands on the pipette will help students hold them steady (one should be close to the base and the other on the handle with a thumb on the button). Instruct them to slowly expel the contents of the pipette, being sure that the tip is not poking through the bottom of the well.

3. Electrophoresis:
   a. Connect the red lead to the positive pole (anode - red) of the battery setup and the black lead to the negative pole (cathode - black) of the batteries.
   b. Be sure to watch for dye movement as the experiment begins to ensure that the apparatus is working properly and that the electrodes have been plugged into the appropriate pole.
   c. When finished, turn the power off first; unplug the power supply from the outlet, and unplug the leads to the electrophoresis chamber.

d. Gingerly move the gel to a disposable plastic tray for staining. Make sure that students wear gloves.

4. Disposal of culture materials:
   a. At the end of the laboratory period, the teacher should collect all bacterial cultures and other instruments that have come into contact with the cultures. Disinfect materials and glassware using a 10% bleach solution.

**Sources of materials:** Carolina Biological Supply Co (catalog # 21-1010)

Table 1. EcoRI and HindIII Fragment size in Base Pairs

| *EcoRI* | *HindIII* |
|---|---|
| Actual bp | Actual bp |
| 21,226 | 23,130 |
| 7,421 | 9,416 |
| 5,804* | 6,557 |
| 5,643* | 4,361 |
| 4,878 | 2,322 |
| 3,530 | 2,027 |
| | 564 |
| | 125 |

*may form a single band

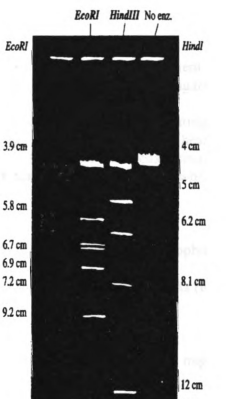



Figure 1. Ideal Restriction Digest of λ DNA

Figure 1. Ideal Restriction Digest of λ DNA

**Problems encountered:** Some of the students' gels produced staircase bands very close to the wells. One reason for this result might have been the lack of buffer on an uneven desk.

**References:**


1. Student's Guide to Exploring Restriction Analysis and Electrophoresis of DNA
2. Teacher's Guide to Exploring Restriction Analysis and Electrophoresis of DNA
3. Advanced Placement BIOLOGY Laboratory Manual for Students Exercises 1-12 Edition D
4. Advanced Placement BIOLOGY Laboratory Manual for Teachers Exercises 1-12 Edition D


**Answers to Questions in the Student Laboratory**

Graph Title:

- Migration Distance of Restriction Fragments

1. For which fragment size(s) was your graph most accurate? For which fragment size(s) was it least accurate? What does this tell you about the resolving ability of agarose-gel electrophoresis?
   - The "best fit" line drawn on the data passes closes to the points representing medium-length fragments. Therefore, the graph was most accurate for these fragments. Points/fragments at either extreme of the range were least accurate. Larger fragments might be resolved on a less concentrated agarose gel, while smaller fragments would resolve on a more concentrated gel.

2. Discuss how each of the following factors would affect the results of electrophoresis:
   a) Voltage used.
      - Increased voltage will increase the rate of migration for all fragments (too high a voltage will melt the gel)

   b) Running time
      - Increased running time will increase the distance that the fragments migrate

   c) Amount of DNA used
      - Increased DNA amounts will increase the intensities of the fragment bands after staining.

   d. Reversal of polarity
      - DNA fragments will move in opposite direction.

3. Two small restriction fragments of nearly the same base-pair size appear as a single band, even when the sample is run to the very end of the gel. What could be done to resolve the fragments? Why would it work?
   - The fragments could be separated on a more concentrated agarose gel. At higher concentration, the pore size of the gel would be more suited to separation of

smaller fragments of similar size. The fragments could be transferred to another electrophoresis chamber that contains a gel other than agarose, such as polyacrylamide. The different affinities between the media and the molecules might allow for a better separation. Another possibility would be to digest the fragments with different restriction enzymes and then utilize the reconstruct of the fragments.

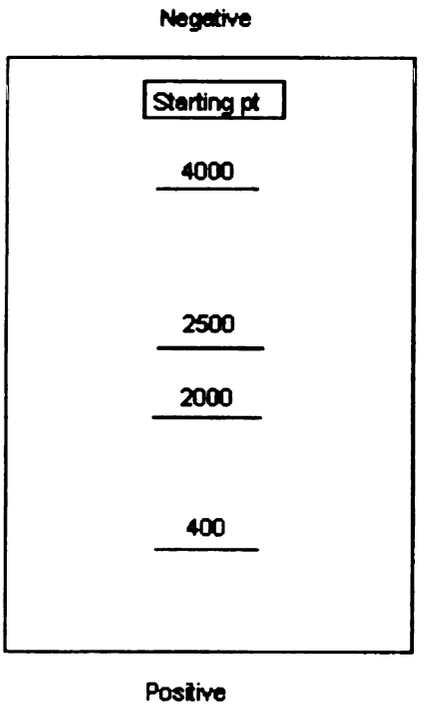
4. What is a plasmid? How are plasmids used in genetic engineering?
   - A plasmid is a circular piece of double-stranded DNA capable of self replicating and being expressed in a bacterial cell. When DNA is spliced into the plasmid a recombinant plasmid is formed. These plasmids may be taken up by bacteria by transformation.

5. What are restriction enzymes? How do they work? What are recognition sites?
   - Restriction enzymes are proteins capable of recognizing a specific DNA sequence (the restriction site) and catalyzing the hydrolysis (cutting) of this DNA molecule.

6. What is the source of restriction enzymes? What is their function in nature?
   - Restriction enzymes are generally isolated from prokaryotic organisms. In nature, they function to protect bacteria from foreign DNA by cutting it into nonfunctional pieces.

7. Describe the function of electricity and the agarose gel in electrophoresis.
   - In electrophoresis, agarose acts to sieve the DNA. The electricity creates a positive charge towards which the negatively charged DNA fragments migrate.

8. If a restriction enzyme digest resulted in DNA fragments of the following sizes: 4,000 base pairs, 2,500 base pairs, 2,000 base pairs, 400 base pairs, sketch the resulting separation by electrophoresis. Show starting point, positive and negative electrodes, and the resulting bands.



9. What are the functions of the loading dye in electrophoresis? How can DNA be prepared for visualization?
   - The loading dye functions to increase the density of the DNA so that it will remain in the wells, and to provide a dye which will run at a more rapid rate thatn the DNA fragments. For visualizaiton, DNA fragments can be stained using methylene blue or other stains.

10. Use the graph you prepared from your lab data to predict how far (in cm) a fragment of 8,000 bp would migrate.
   - A fragment of 8,000bp would migrate a distance between the fragments of 9,416 and 6,557 on the HindIII table (Answers for the standard curve in graph will vary).

11. How can a mutation that alters a recognition site be detected by gel electrophoresis?
   - A point mutation causes a change in the base-pair sequence in the recognition size. The altered sequence cannot be recognized by the active site of the restriction enzyme so that the enzyme will not cut a particular size. Thus a larger fragment will be generated.

# GEL ELECTROPHORESIS OF λ DNA LABORATORY

## INTRODUCTION

### Background Information

Bacteriophages are viruses that invade bacteria. Bacteriophages must take over the molecular machinery of their bacterial hosts to reproduce. The phage lambda (λ) preys upon *Escherichia coli.* It can either multiply within its host destroying it (the lytic cycle), or the λ DNA can insert into the bacterial chromosome and remain dormant there for several generations (the lysogenic cycle). An environmental trigger, such as ultraviolet light, activates the lytic cycle.

Phage λ is a relatively simple organism. It consists of a double-stranded length of DNA wrapped around a core of protein and encapsulated in a proteinaceous coat. The entire genetic makeup (genome) of λ has been sequenced and is 48,502 base pairs long. Within this genome are genes that code for the virus's protein coat, bursting (lysis) of the bacterial cell, integration of λ DNA into the host's chromosome and so on. The order in which these genes are activated is important. For example, it would be of little benefit to the virus if the host bacterial cell were lysed before new virus particles had been assembled. Consequently, λ has evolved an elaborate system of gene regulation that has been studied in great detail.

Relatively little of the λ genome is required to package DNA and deliver it into bacterial cells. About 20,000 base pairs can be deleted from its central region and replaced with DNA from another organism, without affecting the phage's viability. Several specially constructed forms of λ can be used by molecular biologists to transfer new genes into bacteria.

Enzymes that cut up DNA are called restriction endonucleases. Bacteria make them to restrict the proliferation of invading viruses (such as the bacteriophage λ). Different restriction enzymes cut at specific sequences of bases in the DNA. The bacterium's own DNA is protected by the addition of methyl ($-CH_3$) groups to adenine (A) or cytosine (C) bases at the sites that normally are "recognized" by the enzymes. Figure 1 shows recognition sites for three such enzymes.

Restriction enzymes or **restriction endonucleases** are essential tools in recombinant DNA methodology. Several hundred have been isolated from a variety of prokaryotic organisms. Restriction endonucleases are named according to a specific system of nomenclature. The letters refer to the organism (bacteria) from which the enzyme was isolated. The first letter of the name stands for the genus name of the organism. The next two letters represent the second word or the species name. The fourth letter (if there is one) represents the strain of the organism. Roman numerals indicate whether the particular enzyme was the first isolated, the second, or so on.

**Examples:**

EcoRl
E = genus *Escherichia*
co = species *coli*
R = strain RY 13
I =first endonuclease isolated from this bacteria

BamHl
B= *Bacillus*
am = *amyloliquefaciens*
H= strain
I =first endonuclease isolated from this bacteria

Hind111
H = *Haemophilus*
In = *influenzae*
$R_d$ = strain
III= third endonuclease isolated from this bacteria

Restriction endonucleases recognize specific DNA sequences in double-stranded DNA (usually four to six base pair sequences of nucleotides) and digest the DNA at these sites. The result is the production of fragments of DNA of various lengths. Some restriction enzymes cut cleanly through the DNA helix at the same position on both strands to produce fragments with blunt ends (Figure1). Other endonucleases cleave each strand off-center at specific nucleotides to produce fragments with "overhangs" or sticky ends (Figure 2).

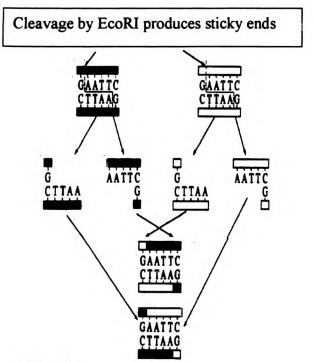


Figure 1

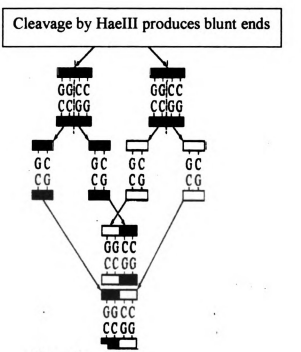
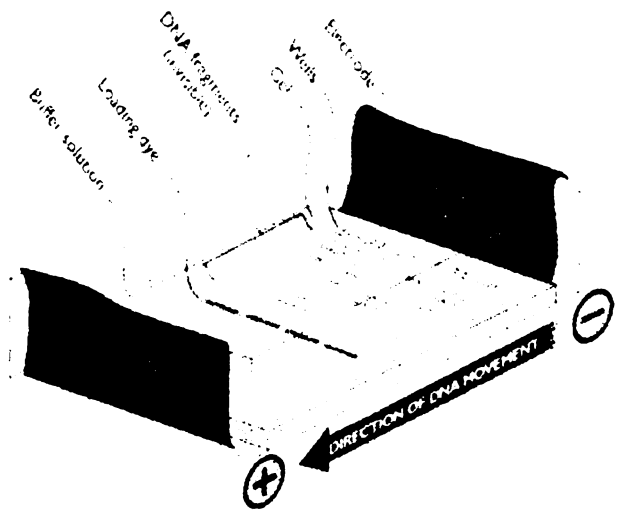


Figure 2

By using the same restriction enzyme to "cut" DNA from two different organisms, complementary "overhangs" or sticky ends will be produced and can allow the DNA from two sources to be "recombined."

**Gel electrophoresis**

Gel electrophoresis can be used to separate DNA fragments of different sizes. First a gel is cast from agarose, a very pure form of polysaccharide, which is obtained from seaweed. At one end of the slab of gel are several small wells, made by the teeth of a comb that was placed in the gel before it sets. A buffer solution is poured over the gel, so that it fills the wells and makes contact with the electrodes at each end of the gel. Ions in the buffer solution conduct electricity. The buffer also stops the gel from drying out.




An electrical current is applied to the electrodes, setting up an electrical field across the gel. When any molecule enters an electrical field, the mobility or speed at which it will move is influenced by the charge of the molecule, the strength of the electrical field, the size and shape the molecule, and the density of the medium (gel) through which it is migrating. Phosphate groups in the backbone of nucleic acids give the DNA fragments a negative electrical charge, so that the DNA migrates through the gel towards the positive electrode. In agarose, the migration rate of linear fragments of DNA is inversely proportional to their size. Small fragments move quickly through the porous gel, whereas larger fragments travel more slowly. In this way the pieces of DNA are separated by size.

The invisible DNA fragments are mixed with a small volume of loading dye. This dye is dissolved in a dense sugar solution, so that when it is added to the wells, it sinks to the bottom, taking the DNA with it. The loading dye/ DNA moves through the gel, so that the progress of the electrophoresis can be seen.

After electrophoresis, the gel is stained to reveal the DNA, either as a smear (many fragments of a wide range of sizes) or bands (each band is comprised of numerous DNA fragments of a similar size). Within a smear, specific bands can by highlighted using probes, which bind to particular sequences of DNA (or RNA). Some probes are radioactive and for reasons of safety cannot be used in schools.

λ DNA, restricted with the enzyme HindIII, is often run on gels alongside other DNA. The λ fragments, which are of known sizes, can then be compared to other DNA fragments, thereby giving an indication of their sizes.

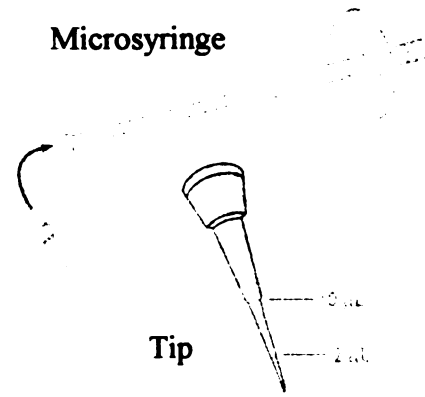
**Objectives**

- Understand how gel electrophoresis separates DNA molecules present in a mixture and use electrophoresis to separate DNA fragments.
- Understand how restriction endonucleases function and demonstrate how they are used
- Understand the importance of restriction enzymes to genetic engineering experiments.
- Determine unknown DNA fragment sizes when given DNA fragments of known size.

**METHOD/ PROCEDURE (Step by step)**

**1. Using the microsyringes**

The microsyringes are designed to be used with disposable tips. Each tip should be used only once, then thrown away. The tips are marked at 2 and 10 microliters (μL), allowing small volumes of liquid to be dispensed with precision. Best results will be obtained if you observe the following precautions:




- Never pull the plunger right out of the microsyringe (when the plunger is re-inserted the seal may become damaged).
- Before you load the microsyringe, pull the plunger out a little (1-2 mm). This will give you some extra air with which to expel the last drop of liquid from the tip.
- When you dispense liquids, hold the microsyringe as near to vertical as possible, and at eye level so that you can see what you are doing.
- Remove the droplet of liquid from the end of the microsyringe tip by touching the inner wall of the microtube.
- Do not touch the point of the microsyringe tip with your fingers. There are proteases and DNases in your sweat, which may contaminate and degrade the reactants.

**2. Rehydrating the dried lambda DNA**

a) Add 100 μL of distilled water to the λ DNA tube (the narrow white tube). Use the 10μL graduation on the tip 10 times to dispense this.

b) Cap the tube lightly and allow it to stand for 5 minutes

c) Hold the closed tube firmly at the top, then flick the side repeatedly with a finger to mix the contents. Do this for one full minute. *If, by accident, you drop the tube or find that drops of liquid are scattered inside the tube, tap it firmly on the bench several times to return the liquid to the bottom.*

*d)* Allow the tube to stand for an additional 5 minutes.

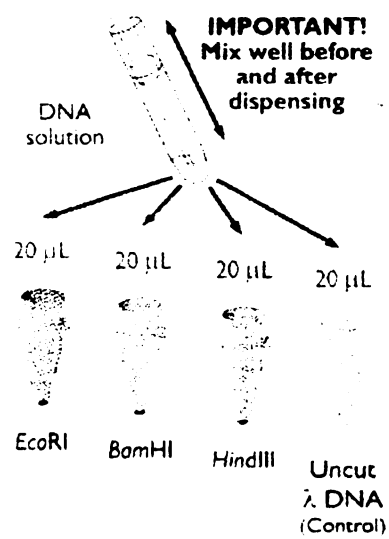
*e)* The λ DNA solution should look slightly opaque. *Note: It is vital that the DNA is thoroughly mixed with the water. To ensure that this is done, always draw the DNA solution up and down in the microsyringe tip a few times.*
*Notes*

- *DNA sticks to glass, For this reason, disposable polypropylene tubes (to which DNA does not adhere) are used in this kit.* The white tube contains 10μg of dried λ DNA. A buffer in the tube helps to maintain the, stability of the DNA once it is in solution. Blue dye has also been added to help you judge when the DNA has dissolved. It is very important to *rehydrate the DNA using the correct volume of water so that the final concentration of the buffer is appropriate for the activity of the enzymes.*

- *A common cause of failure is inadequate mixing of the DNA with water. A "blank" gel usually indicates that the DNA has not been dehydrated at all. Lanes that are overloaded alongside empty ones are often due to uneven mixing of the solution; most of the DNA lies at the bottom of the tube, so it ends up in the last enzyme tube filled.*

**3. Cutting DNA w/ restriction enzymes**

*a)* Add a fresh tip to the microsyringe. Put 20μL of λ DNA solution into an enzyme tube of your choice. Mix the liquid and the dried enzyme by carefully drawing the liquid up and down in the tip a few times. *The liquid in the enzyme tubes should have a distinct blue hue, but there should be no concentration of dye at the bottom of the tube.*




b) Repeat this for each enzyme tube and the yellow "control" tube, using a fresh tip each time to prevent cross-contamination between the tubes.

c) Cap each tube with a matching colored lid.

d) Put the tubes in the foam rack provided (this may be cut into sections, if desired).

e) Incubate the tubes at 37°C, in a water bath or incubator, for between 30 and 45 minutes.

f) Place the tubes in a hot water bath at 65°C for 10 minutes to denature the enzymes.

Notes

- Each tube contains at least 10 units of restriction enzyme. A unit of enzyme will cut 1μg of λ DNA in one hour at 37°C
- The enzyme tubes are color coded:

| *EcoRI* | *BamHI* | *HindIII* | Empty |
|---|---|---|---|
| (Pink) | (blue) | (green) | (yellow) |

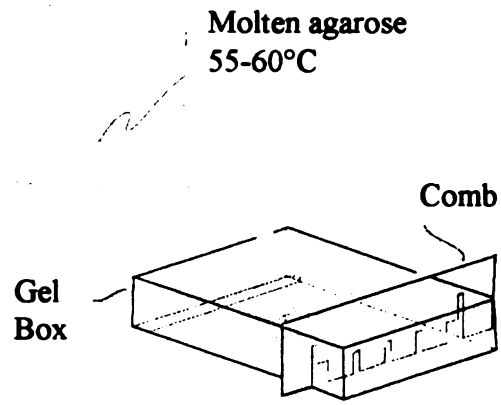
- Like the dried DNA, the enzymes have been preserved with an appropriate buffer and a blue dye. It is essential to add the correct volume of liquid to the tubes, and ensure that their contents are thoroughly mixed. This is because EcoRI and BamHI can be less specific if used in excess or with the wrong concentration of buffer. For instance, in an inappropriate buffer concentration EcoRI may cut DNA molecules at the sequence AATT instead of the longer, more specific sequence, GAATTC,

**4. Preparing the agarose gel**

a) Use a microwave oven, hotplate or a boiling water bath to melt some agarose gel (0.8% made up in TBE buffer). Ensure that no lumps or fibers remain in the molten agarose. Store the molten agarose in a water bath at 55-60°C until it is needed.

b) Place the gel box on a level surface, where you can leave it undisturbed for the next 20-30 minutes. This is necessary because if the gel sets at an angle, the DNA fragments will not run evenly through the gel. Place the comb at one end of the get box.

c) Pour about 10 mL of molten agarose into the gel box so that it fills the central cavity and flows under and between the teeth of the comb. Try not to spill liquid into the areas at either end (if some gel does flow over, just leave it to set-you can scoop it out later).




d) Leave the box, undisturbed, until the get has set hard (agarose gel is opaque when set).

e) Cut two pieces of the carbon fiber tissue, each about 42 mm x 22 mm. These will be the electrodes at either end of the gel box. Put the electrodes to one side until you have loaded the gel.

5. **Loading the gel**

a) Pour slightly more than 10 mL of TBE buffer solution into the gel box. The liquid should just cover the surface of the gel and flood into the areas that will hold the electrodes.

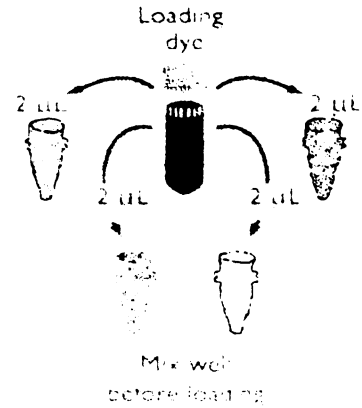
b) Very gently ease the comb from the gel, allowing the buffer solution to fill the wells left behind. Take care not to tear the wells.

c) Put the gel box where it will remain undisturbed while the gel is being "run."

*It is easier to see what you are doing next if the gel box is placed on a dark surface. Alternatively, a strip of black insulating tape can be stuck onto the bottom of the box beneath the wells.*

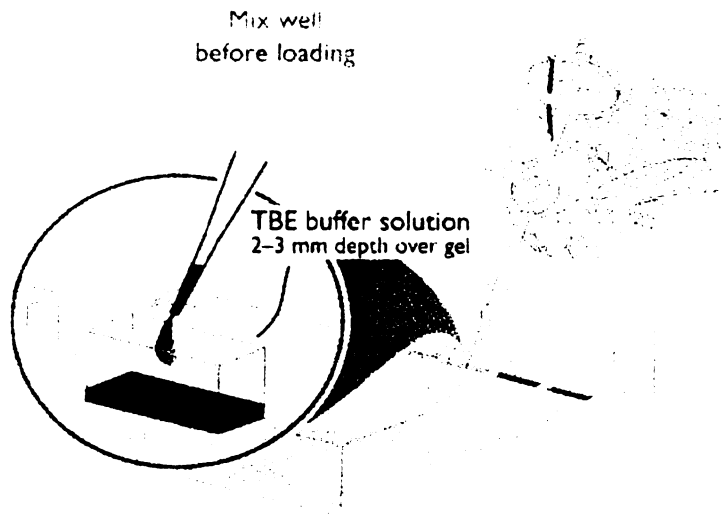
d) Label on the side of the gel box with a marker pen which DNA you will put into the well, include your undigested lambda phage DNA (control).

e) Put a clean tip on the microsyringe. Add 2µL of loading dye to the tube containing the DNA you wish to load. Mix the dye and the DNA sample very thoroughly by drawing the mixture up and down in the microsyringe tip.




*f)* Pipette the loading dye/DNA mixture into one of the labeled wells, holding the tip above the well but under the buffer solution. *The loading dye is denser than the buffer and will move into the well. Take great care not to puncture the bottom of the well with the microsyringe tip.*

*g)* Repeat steps d-f with each DNA digest and the "control" tube with no restriction enzyme. *Remember to use a new tip for each digest, to avoid cross-contamination.*

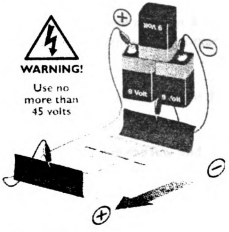



6. **Running the gel**

*a)* Fit one electrode at each end of the box as shown in the illustration. Join the electrodes to batteries using

wires with alligator clips. Sufficient batteries (in series) should be used to give a total voltage of no more than 45 volts. *Ensure that the positive terminal of the battery is connected to the electrode farthest from the wells.*

b) Three nine-volt batteries are used for 3.5 hours. Discard batteries after 2 runs. *Serious or lethal electrical shock could result if the equipment is connected directly to a power supply.*




c) Check that contact is made between the buffer solution and the electrodes (add a little more buffer if necessary). When the current is flowing you should see bubbles on the electrodes. Allow electrophoresis to proceed undisturbed, for several hours. *In a warm room it may be necessary to put the gel box in a plastic bag to reduce evaporation of the buffer.*

d) When the blue loading dye has reached the end of the gel disconnect the batteries. *If you leave the batteries connected, the DNA will run off the end of the gel!*

e) Rinse the alligator clips in tap water and dry them thoroughly to prevent corrosion.

**Notes**

- Ions in the TBE buffer solution used in and above the gel conduct electricity. This buffer solution is alkaline. Under alkaline conditions the phosphate groups of the DNA are negatively charged and therefore the DNA moves towards the positive electrode (anode) when a current is applied. EDTA in the buffer "mops up" or chelates divalent cations, which helps to prevent damage to the DNA because such ions are required (as co- factors) by enzymes that degrade DNA.
- The loading dye does not affect the DNA, but moves through the gel slightly ahead of all but the smallest of DNA fragments (here, those smaller than about 300 base pairs).
- At higher voltages the DNA fragments will move faster, but the separation of bands will not be so clear. This is because the movement of fragments of greater molecular mass is increased differentially at higher voltages, so that they "catch up" with the smaller fragments. At higher voltages the DNA bands may also become distorted.

**7. Staining the DNA**

a) Remove and dispose of the electrodes. Pour off the buffer solution.

b) Wear some plastic gloves to prevent the stain from touching your skin.

*c)* Pour about 10 mL of DNA-staining solution *(Carolina BLU™* concentrate) onto the surface of the gel. Leave it for *exactly* 4 minutes, then return the stain to a bottle for re-use.

*d)* Rinse the surface of the gel very carefully with cold water. Repeat this 3 or 4 times to remove any excess stain. Pour the water away. *Use distilled or deionized water, as this will help to preserve the DNA bands.*

e) Put the gel box in a plastic bag, to prevent the gel from drying out, then leave the gel to "develop" overnight.

*f)* The next day, destain the gel again, this time leaving the water in the box and changing it about 4 times (once every 2 hours). Destaining *will gradually wasb away any "background" stain.*

**Notes**

- Uncut DNA does not move far through the gel, and forms a single, broad band.
- λ DNA cut with a restriction enzyme will form distinct bands of known sizes
- Stained gels may be stored indefinitely in a sealed plastic bag if kept in a refrigerator.

**Picture and diagram:** Determining Fragment Size

a) Examine your stained gel.

b) After observing the gel, carefully wrap it in plastic wrap and smooth out all wrinkles.

c) Using a marking pen, trace the outlines of the sample wells and the location of the bands.

d) Remove the plastic wrap and flatten it out on a white piece of paper on the laboratory bench Save the gel in a Ziplock" plastic bag. Add several drops of buffer. Store at 4'C. You can make your measurements directly from the marked plastic wrap.

## RESULTS

**Background Information:**

The size of the fragments produced by a specific endonuclease (EcoRl in this laboratory) can be determined by using standard fragments of known size (fragments produced by HindIII). When you plot the data on semilog graph paper, the size of the fragments is expressed as the log of the number of base-pairs they contain. This allows data to be plotted on a straight line. The migration distance of the unknown fragments, plotted on the x-axis, will allow their size to be determined on the standard curve.

**Standard Curve for Hind III**

1. Measure the migration distance (in cm) for each HindIII band on your gel. Measure from the bottom of the sample well to the bottom of the band. The migration distance for the largest standard fragment (approximately 23,120 base-pairs) nearest to the origin does not need to be measured. Record these measurements in the Table below.

**Table 1: Distance Hind II produced fragments migrate in agarose gel (cm)**

| Acutal bp | Measured Distance (cm) |
|---|---|
| 23,130 | ---------- --- |
| 9,416 | |
| 6,557 | |
| 4,361 | |
| 2,322 (may form a single band) | |
| 2,027 (may form a single band) | |
| 570 (may not be detected) | |
| 125 (may not be detected) | |

2. Plot the measured migration distance for each band of the standard HindIII digest against the actual base pair (bp) fragment sizes given in Table 1 using the semi-log graph paper.

3. Draw the best-fit line to your points. This will serve as a standard curve.

***B. Interpolated Calculations for EcoRI***

From the standard curve for HindIII, made from known fragment sizes, you can calculate fragment sizes resulting from a digest with EcoRI.

1. Measure the migration distances for each EcoRI band. Record the data in Table 2.

**Table 2: Distance EcoRI produced fragments migrate in agarose gel (cm)**

| Band # | Measured Distance (cm) | Interpolated bp | Actual bp |
|---|---|---|---|
| Band 1 | | | |
| Band 2 | | | |
| Band 3 | | | |
| Band 4 | | | |
| Band 5 | | | |
| Band 6 | | | |

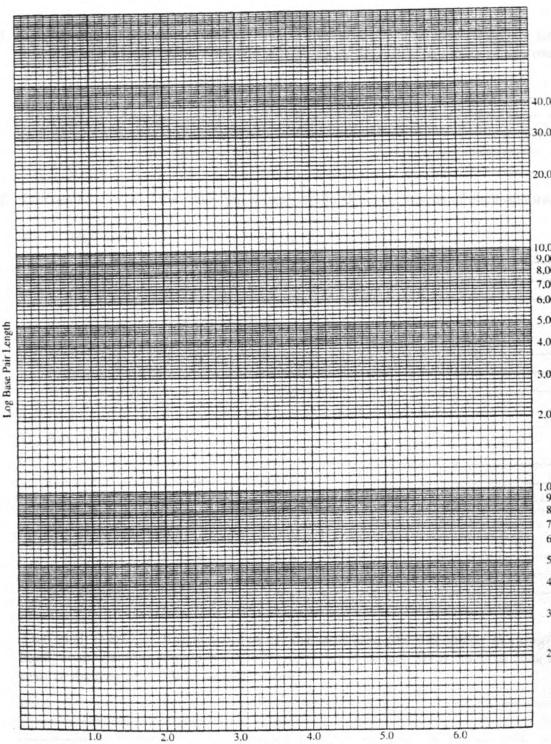
2. **Determine the sizes** of the fragments of phage lambda DNA digested with EcoRI. **Locate on the X-axis** the distance migrated by the first EcoRI fragment. Using a ruler, **draw a vertical line** from this point to its intersection with the best-fit data line. Now **extend a horizontal line** from the intersection point to the Y-axis. This point gives the

base-pair size of this EcoRI fragment. Repeat this procedure and determine the remaining EcoRI fragments. Enter your interpolated data in Table 2.

Your teacher will provide you with the actual base pair data

3. Compare your results to these actual sizes. Note: this interpolation technique is not exact. You should expect as much as 10% to 15% error.

**Graph Title:** _____


40,000
30,000
20,000
10,000
9,000
8,000
7,000
6,000
5,000
4,000
3,000
2,000
1,000
900
800
700
600
500
400
300
200
Log Base Pair Length
1.0
2.0
3.0
4.0
5.0
6.0
Distance Migrated in Cm

## INTERPRETATION AND CONCLUSIONS

### Questions

1. For which fragment size(s) was your graph most accurate? For which fragment size(s) was it least accurate? What does this tell you about the resolving ability of agarose-gel electrophoresis?

2. Discuss how each of the following factors would affect the results of electrophoresis:

   d) Voltage used.

   e) Running time

   f) Amount of DNA used

   e. Reversal of polarity

3. Two small restriction fragments of nearly the same base-pair size appear as a single band, even when the sample is run to the very end of the gel. What could be done to resolve the fragments? Why would it work?

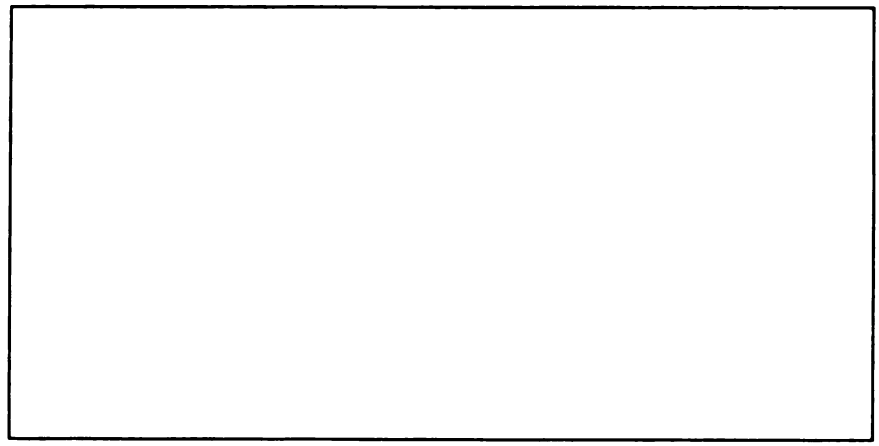
4. What is a plasmid? How are plasmids used in genetic engineering?

5. What are restriction enzymes? How do they work? What are recognition sites?

6. What is the source of restriction enzymes? What is their function in nature?

7. Describe the function of electricity and the agarose gel in electrophoresis.

8. If a restriction enzyme digest resulted in DNA fragments of the following sizes: 4,000 base pairs, 2,500 base pairs, 2,000 base pairs, 400 base pairs, sketch the resulting separation by electrophoresis. Show starting point, positive and negative electrodes, and the resulting bands.



9. What are the functions of the loading dye in electrophoresis? How can DNA be prepared for visualization?

10. Use the graph you prepared from your lab data to predict how far (in cm) a fragment of 8,000 bp would migrate.

11. How can a mutation that alters a recognition site be detected by gel electrophoresis?

## APPENDIX AVIII.

## ALL YOU HAVE ALU LABORATORY

**Instructor's Guide:**

**Target Group:** Advanced Placement Biology students

**Time Frame:** Alu Insertion Polymorphism Kit requires several different activities. Plan your time as follows:

| Day | Time | Activity |
|---|---|---|
| One or more days before lab | 30 min.<br>30 min.<br>40 min. | ✓ ***Pre-Lab:*** Mix and aliquot Chelex<br>✓ ***Pre-lab:*** Set-up work stations<br>✓ ***Pre-Lab:*** Prepare gel solution and cast gels |
| Lab period 1 | 30 min.<br>10 min.<br>70 min. | ✓ Isolate Squamous Cell DNA<br>✓ Set-up PCR reactions<br>✓ Amplify DNA in thermal cycler. |
| Lab period 2 | 15 min.<br>20+ min.<br>10 min. | ✓ Load DNA samples into gels<br>Electrophoresis<br>✓ ***Post-lab:*** Photograph gels |
| Lab period 3 | 40 min | ✓ Results and Discussion |

**Background information for the teacher:**

- ✓ Upon receipt of the kit, store proteinase K, PV92 Primer/Loading Dye Mix, and pBR322/ BstN I markers in **freezer** (approximately -20°C). Other materials may be stored at room temperature (approximately 25°C).
- ✓ Arrange to borrow or use thermal cycle from nearby college. Another method would be to use three separate water baths.
- ✓ If possible arrange an in house field trip and have students do all of the lab in one day.
- ✓ Prior knowledge of basic methods of gel electrophoresis and staining of DNA is presumed.

**Materials:**

- ✓ The materials in the Alu Insertion Polymorphism Kit are sufficient for 25 reactions. The kit includes:
  - Chelex~ 100 resin, 1.5 g
  - Ready-to-Go PCR Beads™, 25* (*Ready-to-Go PCR Beads incorporate Taq polymerase, dNTP's, and $MgCl_2$ Each bead is supplied in an individual O.5-ml test tube.)

- Ready-to-Go PCR Beads™, 25* (*Ready-to-Go PCR Beads incorporate Taq polymerase, dNTP's, and $MgCI_2$ Each bead is supplied in an individual O.5-ml test tube.)
- PV92 Primer/Loading Dye Mix, 700µl
- PBR322/Bst N I markers, .075µg/ml 130µl
- Instructions, student
- Instructions, teacher
- 5 g agarose
- 4 latex gloves

✓ Items of equipment needed but not provided:
- Camera for photographing gels (optional)
- Centrifuge, clinical, for 15 ml tubes, minimum 500 x g
- DNA thermal cycler, programmable
- Electrophoresis chambers for agarose gels
- Electrophoresis power supplies
- Paper cup, 1 per student
- 1.5 ml polypropylene tubes, 2 per student
- 15 ml polypropylene tube, 1 per student
- Micropipets, 1-10µl, 1-20µl, 1-200µl, one for instructor or several shared by students
- Micropipets, 100-1000µl (or 1-ml transfer pipets)
- Forceps, 1
- Laboratory markers, 1 per student
- Micropipet tips, for 1-20µl pipets, 3 per student
- Micropipet tips, for 100-1000µl pipets, 3 per student
- Water bath, boiling, per 12 students

✓ Reagents and supplies needed but not provided:
- Beakers containing ice
- Electrophoresis buffer
- 1µl Ethidium Bromide (stock 0.5) / 1ml buffer
- Saline solution, 0.9% NaCl in water, 10 ml per student

**Prelab Set-up**

✓ Make up a 10% Chelex solution: 1.5g Chelex + 15 ml distilled or deionized water.

✓ For each student, aliquot 500µl of 10% Chelex suspension into a 1.5 ml polypropylene tube. (Be sure to shake the stock tube or draw liquid in and out of pipet tip several times to re-suspend the Chelex beads each time before pipetting a student aliquot).

✓ For each student, prepare and aliquot 10 ml of 0.9% saline solution into a 15ml polypropylene culture tube: 0.9g NaCl per 100 ml distilled or deionized water.

- ✓ PV92 Primer/loading dye mix may collect in tube cap during shipping. To have full volume available for student use, pool reagents by spinning tubes briefly in a microcentrifuge or tapping tube end on desktop.

- ✓ Set up one "boiling water bath per 12 students. This water bath should consist of a beaker and test tube rack to allow 1.5ml centrifuge tubes to be suspended with the reaction in the boiling water. The entire tube should not be submerged.

- ✓ Prepare student stations, each with the following materials:
  - 1-1.5ml polypropylene tube with 500µl of 10% Chelex suspension
  - 1-1.5ml polypropylene tube (empty)
  - Instructions, student
  - Laboratory marker-permanent
  - Micropipet tips
  - Micropipet, 1-10µl or 1-20µl
  - Micropipet, 100-1000µl (or 1-ml transfer pipet)
  - Paper cup
  - Ready-to-Go PCR Bead™ and PCR tube
  - 1-15ml polypropylene tube with 0.9% Saline solution
  - Styrofoam plate turned upside down to hold (1.5ml tubes)

- ✓ Students will share the following materials:
  - Beakers containing ice
  - DNA thermal cycler
  - Forceps
  - PV92 primer/loading dye mix
  - Water bath, boiling
  - Agarose gels w/ Ethidium bromide
  - Camera (optional)
  - Centrifuge, micro
  - PBR3221 BstN I size markers
  - Electrophoresis apparatus
  - Electrophoresis buffer
  - Micropipet tips
  - Micropipet, 1-10µl or 1-20µl
  - Test tube rack (styrofoam plate upside down)
  - Test tubes, 1.5 ml

- ✓ Prepare 1.5% agarose solution sufficient for the number of gels needed to hold all student samples, by adding 5 g agarose to 333 ml of 1 X TBE. Each gel will need to be approximately 8 mm in depth to accommodate samples of to 25µl.
  Program thermal cycler with a step file: (30 cycles link to soak file at 4*C)
  - 94*C – 30 sec
  - 58*C – 30 sec
  - 72*C – 30 sec

**Sources of material:**

- Human Alu Insertion Polymorphism Kit (#21-1232)
  Carolina Biological Supply Company,
  2700 York Rd, Burlington,
  N. Carolina 27215
  1-800-334-5551
- **Deiger catalog**

**Problems encountered:**

I only ordered the PCR kit the first time I tried this lab. Fortunately, I had some left over materials from one of Carolina's electrophoresis labs. Carolina Biological was nice enough to give me the conversions for my extra Carolina BLU final concentrate stain to use for this lab.

- Final Stain = 50ml of final concentrate + 320ml $dH_2O$
- Gel Stain = 1.3g azure blue stain to 1L of $dH_2O$

One of the bands did not show because there wasn't enough DNA (due to pippetting error)

**Reference:**


- Human Alu Insertion Polymorphism Kit (#21-1232)
  Carolina Biological Supply Company,
- Roger Herr, Graduate Student, MSU
- Detection of Alu by PCR,
  http://www.accessexcellence.org/AE/AEPC/DNA/detection.html
- John Gerlich, Assistant Professor, MSU
- Dr. Merle Heideman, Professor, MSU
- Voet & Voet . 2000. Biochemistry

# ALL YOU HAVE ALU LABORATORY

**Background Information**

In this experiment, the polymerase chain reaction (PCR) is used to amplify a short nucleotide sequence from human chromosome 16. The object is to create a personal DNA fingerprint that shows the presence (+) or absence (-) of the Alu transposable element on each chromosome at the PV92 locus. Although DNA from various individuals is more alike than different, many regions of human chromosomes exhibit a great deal of diversity. Such variable sequences are termed "polymorphic" (meaning many forms) and are used for diagnosis of genetic disease, forensic identification, and paternity testing. Many polymorphisms are located in the estimated 95% of the human genome that does not encode proteins.

Alu elements are a component of the non-coding DNA of primate genomes. Alu elements are approximately 300bp in length and derive their name from a single recognition site for the endonuclease Alu I located near the middle of the Alu element. Alu elements are thought to be derived from a gene that encodes the RNA component of the signal recognition particle, which labels proteins for export from the cell.

Alus are transposable DNA sequences that "reproduce" by copying themselves and inserting into new chromosome locations. Alu is an example of "selfish DNA" -it encodes no protein and appears to exist only for its own replication. The human chromosomes contain an estimated 500,000 Alu copies, equaling 5% of the total genome. However, it is thought that, at any point in time, only one or several Alu "masters" are capable of transposing. Each Alu insertion is the "fossil" of a unique transposition event that occurred only once in primate evolution. Thus, all primates sharing an Alu allele are descended from a common ancestor in whom the transposition first occurred.

An estimated 500-2,000 Alu elements are restricted to the human genome. Most Alu mutations are "fixed," meaning that both of the paired chromosomes have an insertion at the same locus (position). However, a number of human-specific Alus recently have been discovered that are dimorphic -an insertion may be present or absent on each of the paired chromosomes of different people. These dimorphic Alus inserted within the last million years, during the evolution and dispersion of modern humans. PCR can be used to screen individuals for the presence or absence of the Alu transposable element.

The source of template DNA for this procedure is a sample of several thousand squamous cells obtained from cheek cells. Cheek cells are obtained by a saline mouthwash, collected by centrifugation, and resuspended in Chelex. Then, the samples are boiled to lyse the squamous cells and liberate the chromosomal DNA. The Chelex binds metal ions, released from the cells that inhibit the PCR reaction. A sample of the clear supernatant, containing chromosomal DNA, is combined with a buffered solution of heat-stable Taq polymerase, oligonucleotide primers, the four deoxynucleotide (dNTP) building blocks of DNA, and the cofactor magnesium chloride ($MgCl_2$). The PCR mixture is placed in a DNA thermal cycler and taken through 30 cycles consisting of:

- 30 seconds at 94°C, while the chromosomal DNA is denatured into single strands,
- 30 seconds at 58°C, while the primers form hydrogen bonds with their complementary sequences on either side of the PV92 locus, and
- 30 seconds at 72°C, while the Taq polymerase makes complementary DNA strands that begin with each primer.

The primers used in the experiment bracket the PV92 locus and result in selective amplification, or copying of that region of chromosome 16. PV92 has only two alleles indicating the presence (+) or absence (-) of the Alu transposable element. Amplification of the (-)allele produces a DNA fragment of 550 bp, while amplification of the (+) allele produces an 850-bp fragment. Alleles of this size can be readily separated by agarose gel electrophoresis. Student amplification products are loaded side-by-side in a 1.5% agarose gel, along with size markers and electrophoresed. After staining with a visible dye, each student can be scored as one of three Alu genotypes (++, +-, or --)

The PV92 insertion polymorphism was specifically selected for use in this laboratory because it is phenotypically neutral. The Alu insertion element does not encode protein. PV92 alleles have no known relationship to disease states, sex determination or any other phenotype. Even though there is no chance of disclosing phenotypic information about the experimenters, the confidentiality of your PV92 genotypes can be maintained by identifying student samples only by numbers.

Visit the DNA Learning Center WWW site (**http://vector.cshl.org/shockan.html**) to view animations on PCR and DNA fingerprinting. This site will be helpful with answering the pre-lab questions.

**OBJECTIVES:**

- understand DNA amplification by polymerase chain reaction
- practice gel electrophoresis technique
- create a personal DNA fingerprint that shows the presence (+) or absence (-) of the Alu transposable element on each chromosome at the PV92 locus.

## PROCEDURE II: ISOLATE CHEEK CELL DNA

**MATERIALS**

- ✓ Laboratory marker
- ✓ 2 - 1.5 ml culture tubes
- ✓ 15ml test tube w/ Saline solution, 0.9%
- ✓ Paper cup
- ✓ Centrifuge (demo desk)
- ✓ 1000µl Micropipet (for 500µl of 10% Chelex)
- ✓ 10% Chelex
- ✓ Floating tube rack
- ✓ Boiling water bath
- ✓ Forceps
- ✓ Beakers containing ice
- ✓ 200µl Micropipet

**METHOD/ PROCEDURE**

1) Use a permanent marker to place your name on two clean 1.5ml tubes and on the 15ml test tube containing 10ml saline (0.9% NaCl) solution.

2) Pour the saline solution into your mouth and vigorously rinse your mouth for 10 seconds. Save test tube for later use.

3) Expel saline solution into the paper cup.

4) Carefully pour saline solution from the paper cup back into the original test tube and close cap tightly. Save paper cup for later use.

5) Place your sample tube, together with other student samples, in a balanced configuration in a clinical centrifuge and spin for 10 minutes.

6) Carefully pour off supernatant into the paper cup. Be careful not to disturb the cell pellet at the bottom of the test tube.

7) Pour supernatant from Step 6 into the sink and rinse down with water.

8) Set micropipet to 500µl. Draw 10% Chelex suspension in and out of the pipet tip several times to suspend the resin beads. Before resin settles, rapidly transfer 500µl of Chelex suspension to the test tube containing your cell pellet.

9) Re-suspend cells by pipetting in and out several times. Examine against light to confirm that no visible clump of cells remain.

10) Transfer 500µl of the resuspended cell pellet and Chelex into a clean 1.5ml reaction tube labeled with your name (on the top).

11) Place your sample in a floating tube rack in the **boiling water bath for 10 minutes**. *Do not submerge or drop the tube into the water.*

12) Use forceps to remove your tube from the boiling water bath and allow cooling for two minutes. The tube may be placed on ice for faster cooling.

*13)* Place your sample tube, with others, in a balanced configuration in the microcentrifuge and spin for 30 seconds to pellet Chelex beads at the bottom of the tube. *Alternately, let tube set for 5 minutes to allow debris to settle.*

14) Use a fresh tip to transfer 200µl of the clear supernatant to a clean 1.5ml tube labeled with your name. Avoid disturbing or transferring any of the Chelex pellet at the bottom of the tube.

15) Store your sample on ice or in the freezer until ready to begin Procedure B.

## PROCEDURE 2: SET UP PCR REACTIONS

**MATERIALS**

- ✓ 2.5µl Micropipet
- ✓ PV92 primer/loading dye mix
- ✓ PCR tube w/ Ready-to-Go PCR Bead™
- ✓ Laboratory marker
- ✓ Beakers containing ice

**METHOD/ PROCEDURE**

1) Ask Teacher to add 22.5µl of PV92 primer/loading buffer mix to a PCR tube containing a Ready- To-Go PCR Bead. Flick tube with finger to dissolve bead.

2) Use fresh tip to add 2.5µl of your DNA to reaction tube, and tap to mix. Pool reagents by pulsing in a microcentrifuge or by sharply tapping tube bottom on lab bench.

3) Label the cap of your tube and side (**Thermal cycler has heated lids**) with a number, as assigned by your teacher. This is to ensure your results will anonymous.

4) Store all samples on ice or in the freezer until ready to amplify according to the following profile.
   - 🌡 94*C – 30 sec
   - 🌡 58*C – 30 sec
   - 🌡 72*C – 30 sec

   (30 cycles link to soak file at 4*C)

## PROCEDURE 3: LOAD AND ELECTROPHORESE PCR PRODUCTS

**MATERIALS**

- ✓ Agarose gels
- ✓ 250ml 1µg/ml ethidum bromide added to the agarose
- ✓ Camera (optional)
- ✓ Centrifuge, micro
- ✓ PBR3221 BstN I size markers
- ✓ Electrophoresis apparatus
- ✓ 150 ml TBA Electrophoresis buffer
- ✓ Loading dye
- ✓ Micropipet tips
- ✓ Micropipet, 1-10µl or 1-20µl
- ✓ Test tube rack
- ✓ Test tubes, 1.5 ml

NOTE: The cresol red and sucrose in the primer mix functions as loading dye, so that amplified samples can be loaded directly into gels.

1) Your instructor will load 20µl of the pBR322-Bst N size markers into one lane of gel.

2) Use a micropipet with a fresh tip to add the entire PCR sample/loading dye mixture (20μl) into your assigned well of a 1.5% agarose gel. *Expel any air from the tip before loading, and be careful not to push the tip of the pipet through the bottom of the sample well.*
3) Electrophorese at 130 volts for about 90 minutes. *Adequate separation will have occurred when the cresol red dye front has moved at least 50 mm from the wells.*
4) Photograph your class results

**RESULTS**

Take a picture of your groups gel and attach it here

## INTERPRETATION AND CONCLUSIONS

### Graphs

1. Observe the photograph of the stained gel containing your sample and those from other students. Orient the photograph with the sample wells at the top. Interpret the band(s) in each lane of the gel:

a) Scan across the photograph to get an impression of what you see in each lane. You should notice that virtually all student lanes contain one to three prominent bands.

b) Now locate the lane containing the pBR322/ BstN I markers. Working from the well, locate the bands corresponding to each restriction fragment: 1,857bp, 1,058 bp, 929 bp, 383 bp, and 121 bp (may be faint).

c) If One band is visible. Compare its migration to that of the 929-bp and 383-bp bands in the pBR322/ BstN I lane. If the PCR product migrates slightly ahead of the 929-bp band, then the person is homozygous for the PV92 Alu insertion (+/+). If the PCR product migrates well behind the 383-bp band, then the person is homozygous for the absence of the PV92 Alu insertion (-/-).

d) If Two bands are visible. Compare migration of each PCR product to that of the 929-bp and 383-bp bands in the pBR322/ BstNl lane. Confirm that one PCR product corresponds to a size of about 850-bp and that the other PCR product corresponds to a size of about 550-bp. The person is heterozygous for the PV92 Alu insertion (+/-).

e) It is common to see an additional band lower on the gel. This diffuse (fuzzy) band is "primer dimer," an artifact of the PCR reaction that results from the primers overlapping one another and amplifying themselves. Primer dimer is approximately 50 bp, and should be in a position ahead of the 121 bp marker. (Note: the presence of primer dimer, in the absence of other bands, confirms that the reaction contained all components necessary for amplification, but that there was insufficient template (DNA) to amplify the PV92 locus.)

f) Additional faint bands, at other positions, occur when the primers bind to chromosomal loci other than PV92 and give rise to "nonspecific" amplification products.

Determine the genotype distribution for the class by counting the number of students of each genotype (+/+, +/- and -/-).

| genotype | Number of classmates |
|---|---|
| +/+ | |
| +/- | |
| -/- | |

## APPENDIX AIX.

## ZEBRAFISH EMBRYO LABORATORY

**Instructor's Guide:**

**Target Group:** Advanced Placement Biology students

**Time Frame:** 3 days

| Day | Time | Activity |
|---|---|---|
| One or more days before lab | 30 min. | Set up tank and add fish |
| Lab period 1 | 30 min.<br>10 min.<br>40 min<br>5 min | ***Pre-lab:*** Set-up work stations<br>Isolate eggs into petri dishes<br>Observe eggs and record<br>label and return eggs |
| Lab period 2 | 50 min | Ss work on interpretation & conclusion |
| Lab period 3 | 55 min | http://visembryo.ucsf.edu/<br>http://chickscope.beckman.uiuc.edu/explore/embryology/ |

**Materials:**

- ✓ 10 gallon fish tank
- ✓ fish (males and females)
- ✓ siphon
- ✓ marbles
- ✓ thermostat
- ✓ fish net
- ✓ disposable pipettes
- ✓ petri dishes
- ✓ microscope slides
- ✓ cover slips
- ✓ disecting microscopes
- ✓ light microscopes

**References:**

Finer, Kim R. 1998. *Internet Activities for General Biology*. Belmont, CA: Wadsworth Publishing Company.
Helm, Biology lab manual 3rd Edition.
http://www.neuro.uoregon.edu/k12/Dev%20Biol%20Lab.html
http://visembryo.ucsf.edu/
http://chickscope.beckman.uiuc.edu/explore/embryology/

## RESULTS

**Answers to Questions in the Student Laboratory:**

Table 1: Early Zebrafish Development Observations and Inferences

| Illustration | Labeled features | Predicted age (in hours) |
|---|---|---|
| **Cleavage** | a. yolk<br>b. blastodisc<br>c. vegetal-pole- nutrients are located (yolk) | 1 hour |
| **Blastula** | a. blastoderm<br>b. animal pole<br>c. | 2.5 hours |
| **Gastrula** | a. epiblast<br>b. hypoblast<br>c. yolk plug | 5 hours |
| **Your Choice** | a. somites<br>b. heart and circulation<br>c. pigmentation in tail | 24 hours |

## INTERPRETATION AND CONCLUSIONS

1. How would you characterize the division pattern of the early cleavages?
   - holoblastic

2. How long is a typical cleavage cell cycle?
   - 15-18 minutes

3. Fill in the following table to summarize the major events of early development.

| | Formation of the Blastula | Early Gastrula | Late Gastrula | Neurula |
|---|---|---|---|---|
| Processes occurring | *cleavage (cell proliferation)* | *gastrulation (migration & invagination)* | *gastrulation (determination & cell differentiation)* | *neurulation (induction & organ differentiation* |
| Type of structure formed | *blastocoel* | *blastopore & archenteron* | *gastrula* | *brain and spinal column* |
| Characteristics of structures | *hollow sphere* | *opening of archenteron* | *archenteron forms* | *neural tube* |
| Significance of stage | *cell positions* | *differentiation of germ layers* | *cells are determined* | *tissues can form organs* |

4. Compare and contrast major developmental events in the sea urchin, zebrafish, and human by completing the following table.

| | Sea Urchin | Frog | Chicken |
|---|---|---|---|
| Type of egg | *isolecithal* | *mesolecithal* | *telolecithal* |
| Pattern of cleavage | *holoblastic (equal)* | *holoblastic (unequal)* | *meroblastic* |
| Distinguishing characteristics of blastula | *uniform, hollow sphere* | *blastocoel towards the animal pole* | *small blastodisc on a large quantity of yolk* |
| How gastrulation occurs | *invagination* | *migration* | *migration* |
| Events of neurulation | *no neurulation* | *indirect development (tadpole develops into adult)* | *direct development (embryo develops directly into adult)* |
| Distinguishing characteristics of later development | *circular blastopore* | *gray crescent, dorsal lip, circular blastopore* | *blastodisc cleavage, primitive streak, elongated blastopore* |

5. What are cytoplasmic determinants?
   - proteins made from stored mRNA that affect early development (up to gastrula), which are produced by maternal genome.

6. How do cytoplasmic determinants affect early development, including cleavage of the zygote?
   - determine the orientation of early clavage planes
   - Ex. controls whether spiral cleavage is to the left or right.

7. What are homeotic genes?
   - genes that control the development of segmentation in organisms

8. How do homeotic genes control the development of segmented organisms?
   - Each homeotic gene contains a conserved homeobox sequence that codes for a DNA regulatory protein that can turn on other needed genes.

9. Describe the function of each of the following extraembryonic membranes in the embryo:
   a. Amnion- filled with amnionic fluid to protect the embryo
   b. Chorion- protection & gas exchange w/ vessels of allantois
   c. Allantois- waste storage & and gas exchange.
   d. Yolk sac- vitelline vessels associated w/ the yolk sac to deliver .....nutrients to the embryo.

10. What is the function of the vitelline blood vessels in the embryo?
    - Vitelline vessels in the chick embryo are found on the surface of the yolk sac. In early development, these vessels assist w/ oxygenation of the blood, picking up oxygen from what has diffused into the shell. Later, the vessels pick up nutrients from the yolk sac. The nutrient laden blood is returned to the heart.

11. In development of the human embryo, the trophoblast is like the chorion, surrounding the embryo in its allantois. The trophoblast becomes the placenta during development of the fetus. How does the function of the human placenta compare to the chorion.
    - The human placenta is responsible for oxygenation of fetal blood & for the removal of wastes and carbon dioxide. In the chick, the chorion/ allantois membrane system carries out the same function. In both the chick and human, the embryo is surrounded by a fluid filled protective amnion.

# ZEBRAFISH EMBRYO LABORATORY

## INTRODUCTION

### Background Information

The heredity material within fertilized eggs, or zygotes, of animal organisms holds the key to both structure and function. DNA guides, through time, the structural and functional development of a single cell into an embryo and its morphogenesis into an adult. Even aging is a result of genetic programming.

Biologists are beginning to understand the genetic control of development. Advanced technologies as well as molecular recombinant DNA techniques have made it possible to discover more and more and more about the genomes of organisms and to map the entire genomes of some. Common patterns of gene function are beginning to emerge, suggesting that some day, perhaps, we will be able to explain the mysteries of animal development.

During this lab you will have the opportunity to explore the development of a simple vertebrate. The zebrafish, *Danio rerio*, is a common aquarium fish that breeds very easily. If fish are maintained in breeding condition, spawning occurs each day at dawn, and with fertilization external, all stages are easily accessible. The embryo and its outer shell are very clear optically, allowing for easy observation throughout its early development. The small size of the embryo allows you to section very deeply into the embryo with slight adjustments of the microscope to reveal much of the internal structural development. Development is also very rapid. The following table gives approximate times (after fertilization) of key developmental stages:

| Stage | Time (28.5oC) |
|---|---|
| 1st cleavage | 0.7 hrs |
| 10th cleavage | (Midblastula) 3 hrs |
| epiboly | 4.3 hrs |
| gastrulation | 5 hrs |
| first movements | 18 hrs |
| heartbeat and pigmentation | 24 hrs |
| swimming | 48 hrs |
| hatching and feeding | 72 hrs |

Part I: Cleavage Period

In order for a fertilized egg or zygote to become a multi-cellular organism, the zygote must divide by mitosis. During early development, this process of division is known as cleavage.

The fertilized egg is not a uniform sphere. Differential concentrations of cytoplasm and yolk (if present) can affect the cleavage process. The upper portion of the

egg, usually richest in cytoplasm, is known as the animal pole, and the lower portion of the egg, containing more yolk, the vegetal pole. The first plane of cleavage is vertical, bisecting both the animal and vegetal poles.

Depending upon the amount of yolk in the egg, the planes of cleavage will differ. If the plane of cleavage passes all the way through the zygote it is referred to as hololblastic cleavage, which is typical of cells with small to medium amounts of yolk (sea urchin and human). On the other hand, if the plane of cleavage only passes a part of the zygote it is called meroblastic cleavage, which is typical of cells with large amounts of yolk (zebrafish and chicken).

A second cleavage division typically occurs at a right angle to the first, producing four cells. The third cleavage division cuts horizontally to form eight cells, four on the top and four on the bottom .The cells produced during these cleavage divisions are known as blastomeres. If the blastomeres in the top tier lie directly above those in the bottom tier, the pattern is said to be radial, a pattern characteristics of echinoderms and chordates.

**OBJECTIVE:**

- ☞ Identify the animal pole and vegetal pole
- ☞ Describe how the amount and distribution of cytoplasm within a fertilized egg influence the patterns of cleavage
- ☞ Determine the cleavage stage of various embryos
- ☞ Describe the important developmental features at each stage

**MATERIALS:**

- ✓ Dissecting microscope
- ✓ Colored pencils
- ✓ Dissecting needle
- ✓ Petri dish
- ✓ 7 coverslips
- ✓ disposable pipette
- ✓ beaker of eggs

**METHOD/PROCEDURE:**

During this period, the first 6 cleavages occur. The cells or blastomeres, divide synchronously at about 15 minutes. Usually, the first five cleavages are all vertical and occur at right angles to one another, as shown in the figure below.

The sample of water in your beaker was obtained by siphoning the bottom of the fish tank in class. In addition to extra food and waste, it should have some zebrafish embryos in early cleavage.

1. With the pipetter, transfer several eggs into the petri dish. Try not to collect a lot of the extra debris. Cover them entirely with the clear water at the top of your beaker to keep them alive.

2. Carefully observe your embryos with the dissecting microscope. IMPORTANT NOTE: Try slight adjustments of your lighting to find the best viewing conditions. Sometimes slightly less lighting allows you to see more detail.

3. Determine the stage of development.

4. Find and transfer a few 2-4 cell embryos to a small petri dish. It will be necessary to change to a monocular scope with greater magnification to see the nuclei. To make this change, you will need to mount the embryo, still in its shell, on a slide in a generous drop of water beneath a coverslip bridge. You do this by stacking 3 coverslips on each side of the embryo and one above it (7 in all). This will prevent the embryo from being squashed. Observe with any objectives except the 100x oil immersion lens.

5. Carefully adjust your lighting to accentuate the nuclei. They are present during the first half of each cycle and their shapes change systematically. The nuclei are globular in very early interphase and become spherical by late interphase. As the cells enter mitosis, the nuclei are elliptical and then take on the shape of a rugby-style football shortly before they disappear during prophase. The mitotic chromosomes are more difficult to visualize. Approximately 5 minutes after the nuclei disappear, you should see cleavage taking place.

6. Gently use the small probe to roll the embryo about the dish. . Take care not to damage the chorion. By observing the cell arrangements from different angles you should be able to figure out the way the cleavages occur. Early cleavages are incomplete, or meroblastic, leaving the yolky region (vegetal pole) of the embryo uncleaved. Orient your embryo so that you are looking down on the animal pole.

7. Follow what is happening during several successive divisions, trying to keep track of more than a single embryo. Notice that divisions often seem to occur along stereotyped "cleavage planes".

8. *Draw sketches* in **Table 1**, but work quickly for things may happen fast while you are not watching.

9. *Record the times* of your observations. You may find that the first 5 or so cleavages occur in a regular, but not an invariant, pattern.

10. Illustrate one of your embryos and indicate the cleavage stage you think it represents in Table 1 below. Your lab partner should draw the other embryo.

11. Find two embryos in the Blastula Period. Follow the same directions as above.

12. Refer to your textbook and other references to describe the characteristic features of your embryo. Record them in Table 1.

## RESULTS

Table 1: Early Zebrafish Development Observations and Inferences

| Illustration | Labeled features | Predicted age (in hours) |
|---|---|---|
| **Cleavage** | | |
| **Blastula** | | |
| **Gastrula** | | |
| **Your Choice** | | |

## INTERPRETATION AND CONCLUSIONS

1. How would you characterize the division pattern of the early cleavages? _____

2. How long is a typical cleavage cell cycle? _____

3. Fill in the following table to summarize the major events of early development.

| | Formation of the Blastula | Early Gastrula | Late Gastrula | Neurula |
|---|---|---|---|---|
| Processes occurring | | | | |
| Type of structure formed | | | | |
| Characteristics of structures | | | | |
| Significance of stage | | | | |

4. Compare and contrast major developmental events in the sea urchin, zebrafish, and human by completing the following table.

| | Sea Urchin | Zebra fish | Human |
|---|---|---|---|
| Type of egg | | | |
| Pattern of cleavage | | | |
| Distinguishing characteristics of blastula | | | |
| How gastrulation occurs | | | |
| Events of neurulation | | | |
| Distinguishing characteristics of later development | | | |

5. What are cytoplasmic determinants?

6. How do cytoplasmic determinants affect early development, including cleavage of the zygote?

7. What are homeotic genes?

8. How do homeotic genes control the development of segmented organisms?

9. Describe the function of each of the following extraembryonic membranes in the embryo:
   - Amnion
   - Chorion
   - Allantois
   - Yolk sac

10. What is the function of the vitelline blood vessels in the embryo?

11. In development of the human embryo, the trophoblast is like the chorion, surrounding the embryo in its allantois. The trophoblast becomes the placenta during development of the fetus. How does the function of the human placenta compare to the chorion.

## APPENDIX BI.

## STUDENT BIOTECHNOLOGY AND DEVELOPMENTAL BIOLOGY SURVEY

1. How would you rate the laboratory experiences in this course in comparison to other science courses?

   *E. coli* Transformation

   [excellent, very good, satisfactory, fairly good, unsatisfactory]

   Gel Electrophoresis

   [excellent, very good, satisfactory, fairly good, unsatisfactory]

   All You Have ALU

   [excellent, very good, satisfactory, fairly good, unsatisfactory]

   Zebrafish Embryo

   [excellent, very good, satisfactory, fairly good, unsatisfactory]

2. How well did the laboratory work relate to the ideas discussed in class?

   [excellent, very good, satisfactory, fairly good, unsatisfactory]

3. How would you rate the classroom simulations of laboratory techniques in terms of helping you to understand biotechnology concepts taught in this course?

   Breakfast DNA--> protein

   [excellent, very good, satisfactory, fairly good, unsatisfactory]

   Manipulating DNA Replication

   [excellent, very good, satisfactory, fairly good, unsatisfactory]

4. How would you rate the Medical Technology and Genetic Testing Field Trip to MSU in terms of interest and usefulness to the study of biology?

   [excellent, very good, satisfactory, fairly good, unsatisfactory]

5. How do you rate the way you were graded on labs, tests, and essays in this course?

   [excellent, very good, satisfactory, fairly good, unsatisfactory]

6. How would you rate the laboratory classroom environment (how well students know, help and are support one another) ?

   [excellent, very good, satisfactory, fairly good, unsatisfactory]

7. How would you rate the interpersonal behavior of the teacher?

   [excellent, very good, satisfactory, fairly good, unsatisfactory]

## APPENDIX BII.

## DNA STRUCTURE AND REPLICATION PRETEST

Use the figure below to answer questions 1-5.

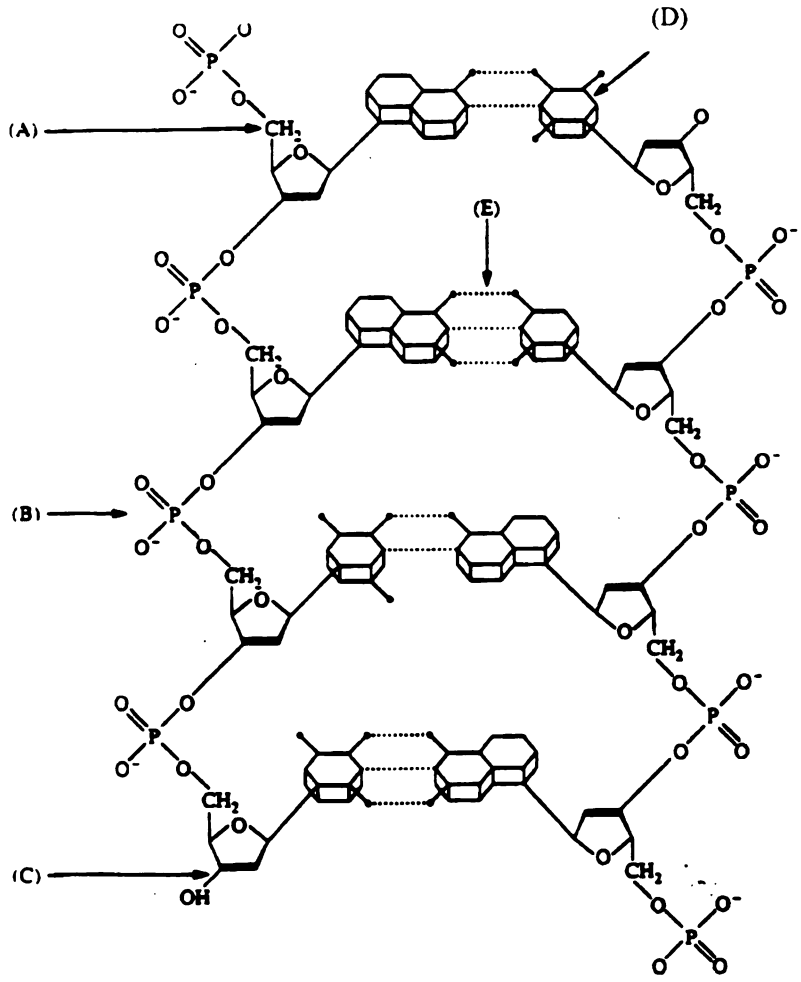
1. A chemical group that, together with a sugar and a nitrogen base, makes up a nucleotide

2. A hydrogen bond

3. A pyrimidine

4. A 5' carbon of deoxyribose

5. Most likely to be broken during replication



6. DNA replication can be described as
   a. semiconservative
   b. conservative
   c. degenerative
   d. dispersive
   e. radical

7. In DNA replication, DNA polymerase catalyzes the reaction in which:
   a. the double helix unwinds
   b. the sugar-phosphate bonds of each strand are broken
   c. a phosphate group is added to the 3'-carbon or 5'-carbon of ribose
   d. a nucleotide with a base complementary to the base on the template strand is added to the new DNA strand
   e. the two nucleotide strands come together and intertwine to form a double helix

**Answer Key:**

1. b
2. e
3. d
4. a
5. e
6. a
7. d

## DNA STRUCTURE AND REPLICATION POST-TEST

Use the figure below to answer questions 1-5.

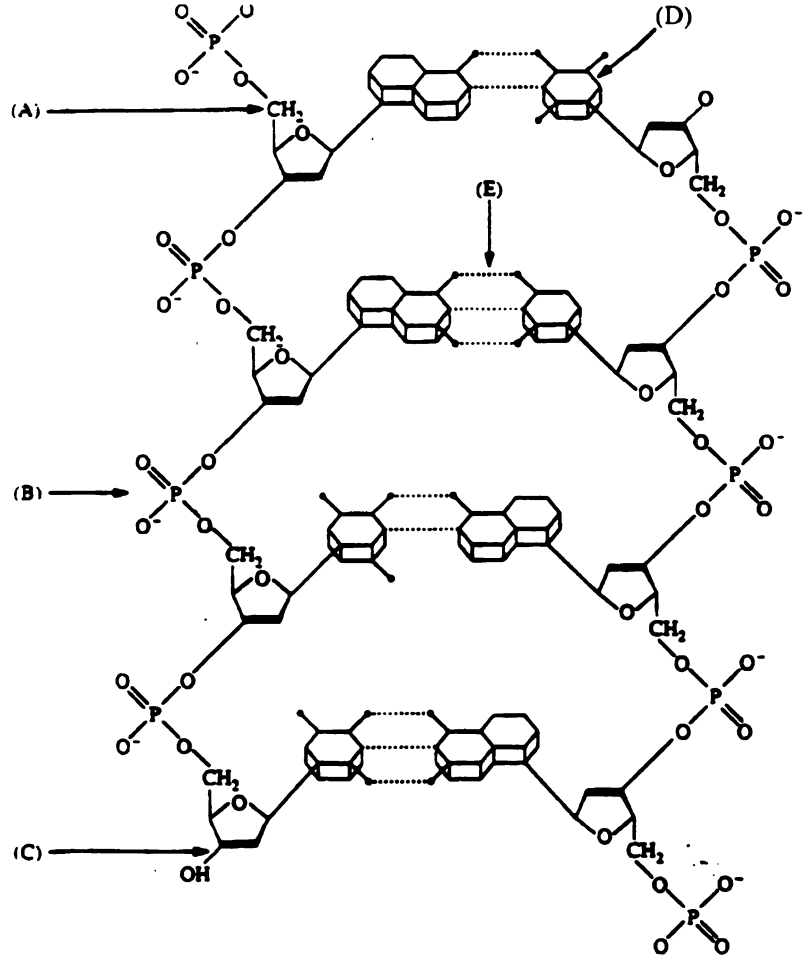
1. A chemical group that, together with a sugar and a nitrogen base, makes up a nucleotide

2. A hydrogen bond

3. A pyrimidine

4. A 5' carbon of deoxyribose

5. Most likely to be broken during replication



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   a. semiconservative
   b. conservative
   c. degenerative
   d. dispersive
   e. radical

7. In DNA replication, DNA polymerase catalyzes the reaction in which:
   a. the double helix unwinds
   b. the sugar-phosphate bonds of each strand are broken
   c. a phosphate group is added to the 3'-carbon or 5'-carbon of ribose
   d. a nucleotide with a base complementary to the base on the template strand is added to the new DNA strand
   e. the two nucleotide strands come together and intertwine to form a double helix

8. DNA contains all of the following molecules EXCEPT:
   a. adenine
   b. guanine
   c. deoxyribose
   d. phosphate
   e. uricil

9. "Primers" that initiate DNA replication consist of
   a. RNA nucleotides
   b. DNA nucleotides
   c. Okazaki fragments
   d. DNA polymerase
   e. nucleosomes

10. The two strands of a DNA molecule are connected by
    a. hydrogen bonds between the codons and anticodons
    b. hydrogen bonds between the bases of one strand and the bases of the second strand
    c. hydrogen bonds between deoxyribose sugar molecules of one strand and deoxyribose molecules of the second strand
    d. covalent bonds between phosphate groups
    e. covalent bonds between the nitrogen bases

11. All of the following combinations of nucleotides are examples of normal base pairing EXCEPT
    a. an adenine DNA nucleotide to a thymine DNA nucleotide
    b. a guanine DNA nucleotide to a cytosineDNA nucleotide
    c. a thymine DNA nucleotide to an adenine DNA nucleotide
    d. a cytosine DNA nucleotide to a guanineDNA nucleotide
    e. a uricil RNA nucleotide to a thymine DNA nucleotide

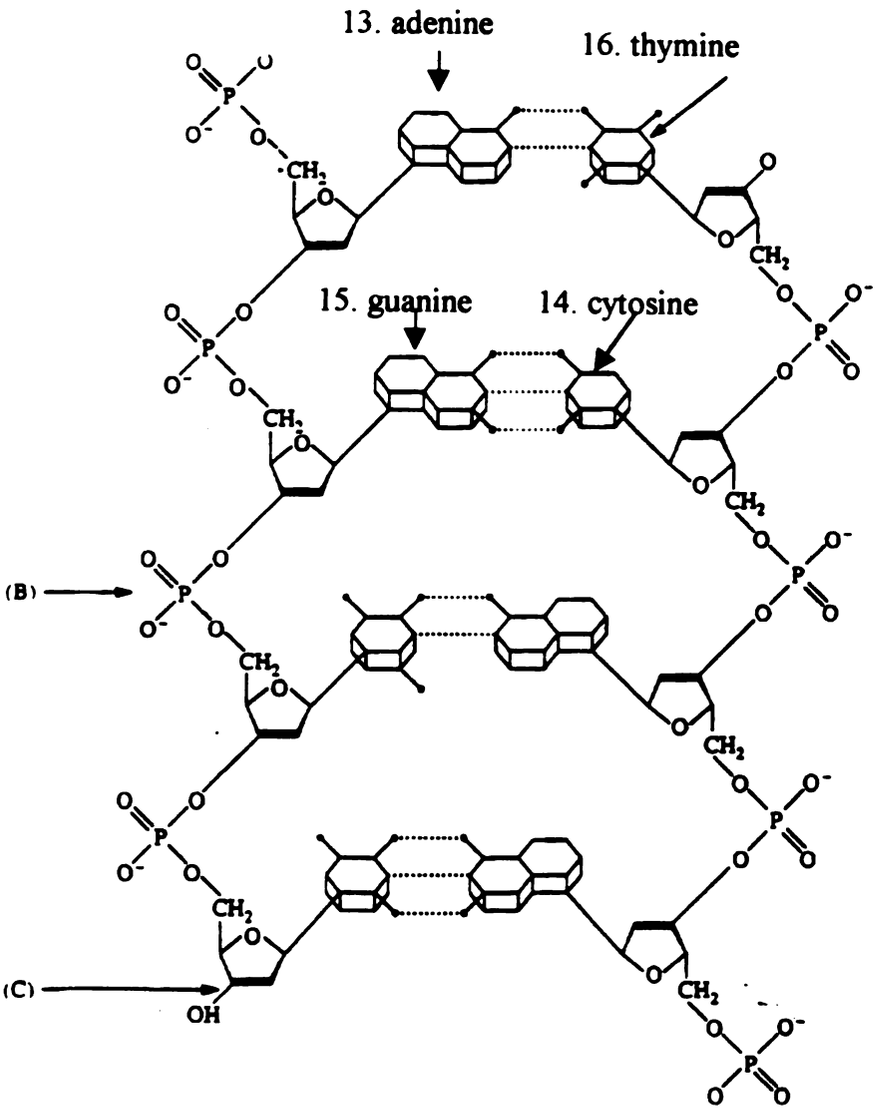
12. All of the following enzymes are involved in DNA replication EXCEPT
    a. helicase
    b. DNA ligase
    c. DNA polymerase
    d. RNA polymerase
    e. primase

Questions 13-16 refer to the diagram of DNA on the first page.

13. Label adenine
14. Label cytosine
15. Label guanine
16. Label thymine

**Answer Key:**

1. b
2. e
3. d
4. a
5. e
6. a
7. d
8. e
9. a
10. b
11. c
12. d



## APPENDIX BIII.

## BACTERIAL TRANSFORMATION PRE AND POST-TEST

1. Once a plasmid has incorporated specific genes such as the gene coding for the antibiotic ampicillin, into its genome, the plasmid may be cloned by
   a. inserting it into a virus to generate multiple copies
   b. treating it with a restriction enzymes in order to cut the molecule into small pieces
   c. inserting it into a suitable bacterium in order to produce multiple copies
   d. running it on a gel electrophoresis in order to determine the size of the gene of interest
   e. infecting it with a mutant cell in order to incorporate the gene of interest

2. A bacteriophage is a
   a. bacterium that atttacks viruses
   b. virus that attacks bacteria
   c. bacterium that attacks eukaryotic cells
   d. parasitic bacterium
   e. parasitic eukaryotic cell

3. Which of the following correctly describes plasmids?
   a. They are composed only of RNA
   b. They are composed of RNA and protein
   c. They are DNA segments in the chromosomes of bacteria
   d. They are the DNA cores of viruses
   e. They can be transferred between bacteria during conjugation

4. In bacteria, a small circle of DNA found outside the main chromosome is called a
   a. Plasmid
   b. cDNA
   c. RFLP
   d. PCR
   e. Genetic fingerprint

5. All of the following cell components are found in prokaryotic cells EXCEPT
   a. DNA
   b. Ribosomes
   c. Cell membranes
   d. Nuclear envelope
   e. Enzymes

6. Which of the following statements about plasmids is correct?
   a. They are synthesized in the endoplasmic reticulum
   b. They are found only in eukaryotic cells
   c. They are composed of RNA
   d. They are larger in size than bacterial chromosomes
   e. They are self-replicating.

7. A plasmid
   a. can be used as a DNA vector
   b. is a type of bacteriophage
   c. is a type of cDNA
   d. is a retrovirus
   e. both b and c

8. DNA molecules with complementary "sticky ends" associate by
   a. covalent bonds
   b. hydrogen bonds
   c. ionic bonds
   d. disulfide bonds
   e. phosphodiester linkages

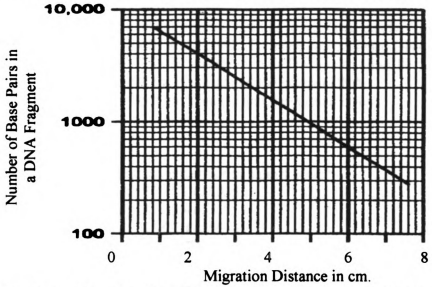
**Answer Key:**

1. c
2. b
3. e
4. a
5. d
6. e
7. a
8. b

## APPENDIX BIV.

## GEL ELECTROPHORESIS PRE AND POST-TEST

Questions 1-3 refers to the following semi-log graph of results from the standard restriction enzyme used in a gel electrophoresis procedure. Phage lambda DNA molecules digested with HindIII are used as the standard.




1. A fragment of phage lambda DNA produced by EcoRI endonuclease migrates 6 cm. If this fragment is produced during the same electrophoresis procedure as the standard shown in the graph above, how large is the fragment?
   a. 150 base pairs
   b. 600 base pairs
   c. 800 base pairs
   d. 1000 base pairs
   e. 6000 base pairs

2. What is the relationship between migration distance and DNA fragment size?
   a. migration distance is independent of DNA fragment size
   b. longer DNA fragments travel a greater distance than shorter fragments
   c. migration distance is inversely proportional to the fragment size.
   d. Migration distance is directly proportional to the fragment size.
   e. The heavier the fragments size the greater the migration distance.

3. According to the graph, approximately how far will a DNA fragment with 3000 base pairs travel on the gel.
   a. 1.0 cm
   b. 2.0 cm
   c. 2.3 cm
   d. 2.6 cm
   e. 5.0 cm

4. Human DNA and a particular plasmid both have sites that can be cut by the restriction enzymes HindIII and EcoRI. To make recombinant DNA, one should
   a. cut the plasmid with EcoRI and the human DNA with Hind III
   b. use EcoRI to cut both the plasmid and the human DNA
   c. use Hind III to cut both the plasmid and the human DNA
   d. a or b
   e. b or c

5. Which of the following sequences is not palindromic?
   a. 5'-AAGCTT-3' & 3'-TTCGAA-5'
   b. 5'-GATC-3' & 3'-CTAG-5'
   c. 5'-GAATTC-3' & 3'-CTTAAG-5'
   d. 5'-CTAA-3' & 3'-GATT-5'

6. Gel electrophoresis separates nucleic acids on the basis of differences in
   a. length
   b. charge
   c. nucleotide sequence
   d. relative proportions of adenine and guanine
   e. relative proportions of thymine and cytosine

The diagram below shows a segment of DNA with a total length of 4,900 base pairs. The arrows indicate reaction sites for two restriction enzymes (enzyme X and enzyme Y).

| | Enz X | Enz Y | Enz X | Enz X | |
|---|---|---|---|---|---|
| **DNA Segment** | I---V | ---V | ----------V | ----------V | ----------I |
| **Length (base pairs)** | 400 | 500 | 1200 | 1300 | 1500 |

7. Explain how the principles of gel electrophoresis allow for the separation of DNA fragments

8. Describe the results you would expect from electrophoretic separation of fragments from the following treatments of the DNA segment above. Assume that the digestion occurred under appropriate conditions and went to completion

   a. DNA digested with only enzyme X

   b. DNA digested with enzyme X and Y

**Answer Key:**

1. b
2. c
3. d
4. e
5. d
6. a

7. 1pt each with a 4 points max.
   - Electricity - Electrical potential (charge, field) moves fragments
   - Charge - Negatively charged fragments move toward (+) anode through gel (-) charge due to phosphate groups
   - Rate/size - Smaller fragments move faster (farther) relative to larger fragments. Describe logarithmic relationship
   - Calibraton - DNAs of known molecular weights are used as markers/standards
   - Apparatus - DNA is stained for visualization of bands/explains use of wells, gel material, tracking dye, buffers.

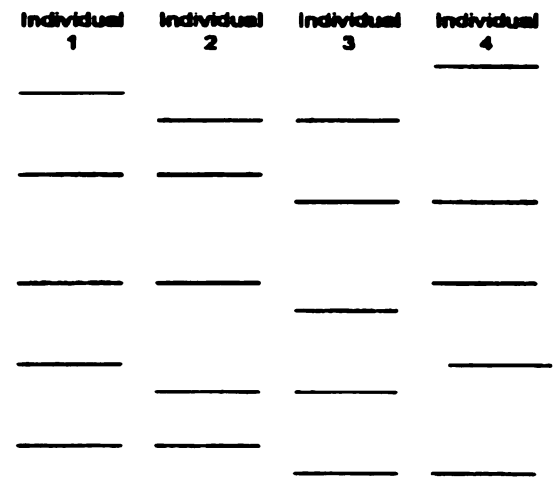
8. 1pt each with a 4 points max.
   a. DNA digested with only enzyme X
      - Describes 400, 1300, 1500, 1700,bp fragments
      - correct 4 band diagram

   b. DNA digested with enzyme X and Y
      - Describe 400, 500, 1200, 1300, 1500 bp fragments
      - correct 5 band diagram

## APPENDIX BV.

## POLYMERASE CHAIN REACTION PRE AND POST-TEST

Question 1 refers to the following diagram representing the bands produced by an electrophoresis procedure using DNA from four human individuals. Each DNA sample is treated with the same restriction enzyme.




1. Which of the following is a correct interpretation of the gel electrophoresis data?
   a. Individual 1 could be an offspring of individuals 3 & 4.
   b. Individual 1 could be an offspring of individuals 2 & 3.
   c. Individual 2 could be an offspring of individuals 1 & 3.
   d. Individual 3 could be an offspring of individuals 2 & 4.
   e. All four individuals could be the same people.

Question 2 refers to the following diagram of a DNA segment


2. Arrows in the above diagram show the cleavage points for the restriction enzyme *TaqI*. Which of the following describes the correct DNA segments produced after treatment with the restriction enzyme?

   a. two fragments: 5'-TCT and CGACT-3'<br>3'-AGAGC TGA-5'

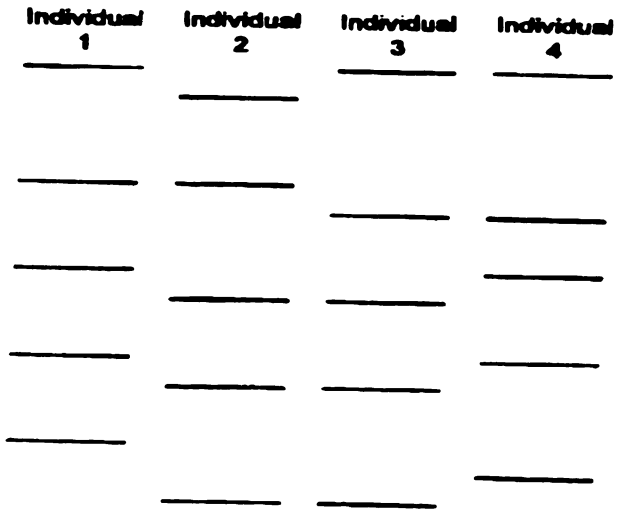
   b. two fragments: 5'-TCTCGACT-3'<br>3'-AGAGCTGA-5'

   c. two fragments: 5'-TCTCGTGA-3'<br>3'-AGAGCACT-5'

   d. four fragments: 5'-TCT, 3'-AGAGC, CGACT-3', and TGA-5'

   e. four fragments: 5'-TCTCG, ACT-3', 3'-AGAGC, and TGA-5'

Question 3 refers to the following diagram. DNA material from each of four individuals was treated with a restriction enzyme. The products were separated using gel electrophoresis. The results, which show relative migration distances for DNA fragments from each individual, are given below.




3. Which of the following is a correct interpretation of the gel electrophoresis data?
   a. all four individuals could be the same person
   b. Individual 3 could be an offspring of individuals 2 and 4.
   c. Individual 2 could be an offspring of individuals 1 and 3.
   d. Individual 1 could be an offspring of individuals 2 and 3.
   e. Individual 1 could be an offspring of individuals 3 and 4.

4. The PCR technique uses
   a. heat-resistant DNA polymerase
   b. reverse transcriptase
   c. DNA ligase
   d. A nd b
   e. B and c

5. In restriction fragment length polymorphism (RFLP) analysis to determine parentage
   a. every band present in a child would be expressed to be present in both of the true parents
   b. every band present in a child would be expected to be present in at least one of the true parents
   c. every band present in a true parent would be expected to be present in all of the children
   d. a and b
   e. b and c

6. Why is the PCR technique valuable?

   _____

   _____

7. Describe the major steps of PCR.

   _____

   _____

   _____

   _____

**Answer Key:**

1. d
2. a
3. c
4. a
5. b

6. Polymerase chain reaction (PCR) is a method of greatly increasing the quantity of a specific DNA or RNA sequence.

7. 1pt each with a 3 points max.
- The double-stranded DNA to be amplified is first heated to separate the two strands.
- Synthetic primers are bound to separated strands. The primers define the ends of the sequences to be amplified.
- The two new strands are synthesized with DNA polymerase extending the strands from the primers giving complementary strands.
- Each cycle is repeated 30 times, doubling the amount of DNA each time.

**Answer Key:**

1. d
2. a
3. c
4. a
5. b

6. Polymerase chain reaction (PCR) is a method of greatly increasing the quantity of a specific DNA or RNA sequence.

7. 1pt each with a 3 points max.
- The double-stranded DNA to be amplified is first heated to separate the two strands.
- Synthetic primers are bound to separated strands. The primers define the ends of the sequences to be amplified.
- The two new strands are synthesized with DNA polymerase extending the strands from the primers giving complementary strands.
- Each cycle is repeated 30 times, doubling the amount of DNA each time.

## APPENDIX BVI.

## DEVELOPMENTAL BIOLOGY PRE AND POST-TEST

1. In human females fertilization normally occurs in the
   a. ovary
   b. uterus
   c. vagina
   d. oviduct
   e. cervix

2. In mammalian embryo, the somites give rise to the notocord
   a. the central nervous system
   b. the lining of the mouth
   c. the eyes
   d. muscles and vertebrae

3. Gastrulation results in three primary tissue layers that give rise to all the organs and tissues of the body. Which of the following statements is true.
   a. Endoderm gives rise to muscle.
   b. Epiderm gives rise to skin
   c. Mesoderm gives rise to bone
   d. Ectoderm gives rise to the gut lining
   e. Periderm gives rise to skin

4. Embryonic induction is best illustrated by which of the following:
   a. Formation of the lens in the ectoderm after contact with the underlying optic cup
   b. Replacement of cartilage by bone
   c. Lysosomal action on the degenerating tail of a tadpole
   d. Development of the amnion surrounding the embryo
   e. Development of the mesoderm into the notochord

5. Which of the following describes the correct sequence of stages during embryogenesis:
   a. Cleavage, blastula formation, gastrulation
   b. Cleavage, gastrulation, blastula formation
   c. Blastula formation, gastrulation, cleavage
   d. Blastula formation, cleavage, gastrulation
   e. Gastrulation, cleavage, blastula formation

Questions 6-9 refer to the following choices

a. Cleavage
b. Organogenesis
c. Gastrulation
d. Neurulation
e. Fertilization

6. This process establishes the primary germ layers.

7. Two haploid cells fuse to form a diploid cell.

8. The number of cells increases, but there is no increase in total cell mass and there is little or no differentiation

9. Cells migrate over the dorsal lip of the blastopore.

10. In animals, all of the following are associated with embryonic development EXCEPT
    a. migration of cells to specific areas
    b. formation of germ layers
    c. activation of all the genes in each cell
    d. inductive tissue interactions
    e. cell division at a relatively rapid rate

11. In humans primary oocytes are located in the
    a. cervix
    b. uterus
    c. corpus luteum
    d. oviduct
    e. ovary

12. All of the following statements about the placenta are correct EXCEPT
    a. It permits an interchange of $CO_2$ and $O_2$ between maternal and fetal blood.
    b. It forms from tissues of both the embryo and the uterus.
    c. It permits the mixing of maternal and fetal blood.
    d. It functions as an endocrine gland.
    e. It provides the embryo with a way to dispose of its nitrogenous waste products.

**Answer Key:**

| | |
|---|---|
| 1. d | 7. e |
| 2. e | 8. a |
| 3. c | 9. d |
| 4. a | 10. c |
| 5. a | 11. e |
| 6. c | 12. c |

# APPENDIX CII.

**Table 2: INDIVIDUAL STUDENTS' PRE AND POST-TEST RESULTS**

| Student | Develop | Develop | DNA Structure | DNA Structure | DNA Replication | DNA Replication | Trans-formation | Trans-formation |
|---|---|---|---|---|---|---|---|---|
| | Pre=12pts | Post=12pts | Post=4pts | Post=4pts | Pre= 3pts | Post= 3pts | Pre= 8pts | Post=8pts |
| a | 1 | 7 | 1 | 3 | 1 | 3 | 2 | 6 |
| b | 1 | 9 | 2 | 3 | 0 | 2 | 1 | 5 |
| c | 2 | 10 | 0 | 4 | 2 | 3 | 2 | 8 |
| d | 0 | 5 | 1 | 4 | 1 | 2 | 3 | 6 |
| e | 1 | 8 | 0 | 4 | 1 | 3 | 2 | 8 |
| f | 0 | 6 | 2 | 4 | 0 | 2 | 2 | 8 |
| g | 0 | 6 | 1 | 3 | 0 | 1 | 2 | 8 |
| h | 2 | 9 | 1 | 4 | 1 | 2 | 1 | 7 |
| I | 2 | 9 | 0 | 3 | 1 | 3 | 2 | 8 |
| j | 2 | 8 | 1 | 3 | 1 | 2 | 2 | 7 |
| k | 1 | 2 | 0 | 4 | 0 | 2 | 1 | 5 |
| l | 0 | 9 | 1 | 4 | 1 | 1 | 2 | 5 |
| m | 0 | 7 | 1 | 3 | 0 | 2 | 2 | 7 |
| n | 0 | 10 | 2 | 3 | 1 | 3 | 2 | 8 |
| o | 0 | 8 | 1 | 3 | 1 | 2 | 1 | 6 |
| p | 4 | 12 | 0 | 4 | 1 | 3 | 2 | 8 |
| q | 0 | 9 | 1 | 4 | 1 | 3 | 2 | 6 |
| r | 1 | 8 | 0 | 2 | 0 | 3 | 1 | 7 |
| s | 1 | 8 | 1 | 4 | 1 | 3 | 1 | 4 |
| t | 0 | 10 | 0 | 4 | 1 | 3 | 2 | 8 |
| Total | 18 | 160 | 16 | 70 | 15 | 48 | 35 | 135 |
| Ave | 0.900 | 8.000 | 0.800 | 3.500 | 0.750 | 2.400 | 1.750 | 6.750 |
| Ave % | 7.5 | 66.67 | 20.00 | 87.50 | 25.00 | 80.00 | 21.88 | 84.38 |

| Student | Electrophoresis (MC) | | Electrophoresis (SA) | | PCR (MC) | PCR (MC) | PCR (SA) | PCR (SA) |
|---|---|---|---|---|---|---|---|---|
| | Pre=6pts | Post=6pts | Pre=7pts | Post=7pts | Pre= 5pts | Post= 5pts | Pre=4pts | Post=4pts |
| a | 1 | 5 | 0 | 3.5 | 1 | 4 | 0 | 3.5 |
| b | 4 | 6 | 2 | 3 | 2 | 4 | 0 | 1 |
| c | 2 | 6 | 0 | 6.5 | 1 | 5 | 0 | 4 |
| d | 1 | 5 | 0 | 3 | 2 | 5 | 0 | 3 |
| e | 2 | 6 | 0 | 3 | 1 | 4 | 0 | 3.5 |
| f | 1 | 5 | 0 | 0 | 2 | 4 | 0 | 3 |
| g | 4 | 6 | 0 | 2.5 | 1 | 4 | 0 | 2 |
| h | 2 | 6 | 0 | 2 | 1 | 5 | 0 | 4 |
| I | 2 | 6 | 0 | 5 | 1 | 5 | 0 | 3 |
| j | 3 | 6 | 0 | 3.5 | 1 | 5 | 0 | 4 |
| k | 3 | 6 | 0 | 1 | 0 | 4 | 0 | 0 |
| l | 1 | 2 | 0 | 3.75 | 1 | 5 | 0 | 3.5 |
| m | 2 | 6 | 0 | 1.5 | 1 | 5 | 0 | 2.5 |
| n | 2 | 6 | 0 | 4 | 2 | 5 | 0 | 3 |
| o | 3 | 6 | 0 | 2.5 | 0 | 4 | 0 | 3 |
| p | 2 | 5 | 0 | 4 | 2 | 5 | 0 | 3 |
| q | 4 | 6 | 0 | 5 | 1 | 4 | 0 | 3 |
| r | 2 | 5 | 0 | 4 | 1 | 5 | 0 | 1.5 |
| s | 1 | 5 | 0 | 3.5 | 1 | 5 | 0 | 3 |
| t | 3 | 4 | 0 | 3.5 | 0 | 5 | 0 | 4 |
| Total | 45 | 108 | 2 | 64.75 | 22 | 92 | 0 | 57.5 |
| Ave | 2.250 | 5.400 | 0.100 | 3.238 | 1.100 | 4.600 | 0.000 | 2.875 |
| Ave % | 37.5 | 90 | 1.43 | 46.25 | 22.00 | 92.00 | 0.00 | 71.88 |

## APPENDIX CIII.

### Table 3: T-TEST RESULTS FOR ALL PRE AND POST-TESTS

| Test | Total points possible | Pre-test Mean | Post-test Mean | t- Test probability | Inverse of t distribution |
|---|---|---|---|---|---|
| | | | | | |
| Embryology | 12 | 0.90 | 8.00 | 1.62E-13 | 5000000 |
| DNA Structure | 4 | 0.80 | 3.50 | 1.66E-15 | 5000000 |
| DNA Replication | 3 | 0.75 | 2.40 | 4.39E-10 | 5000000 |
| Transformation | 8 | 1.75 | 6.75 | 8.23E-15 | 5000000 |
| Gel Electrophoresis (MC) | 6 | 2.25 | 5.40 | 4.67E-12 | 5000000 |
| Gel Electrophoresis (SA) | 7 | 0.10 | 3.24 | 4.36E-09 | 5000000 |
| PCR (MC) | 5 | 1.10 | 4.60 | 1.67E-20 | 5000000 |
| PCR (SA) | 4 | 0.00 | 2.83 | 3.25E-10 | 5000000 |

## APPENDIX CIV.

### Table 4: AVERAGE PRE AND POST-TEST PERCENTAGES

| Test | Pre Ave % | Post Ave % |
|---|---|---|
| Embryology | 7.50 | 66.67 |
| DNA Structure | 20.00 | 87.50 |
| DNA Replication | 25.00 | 80.00 |
| Transformation | 21.88 | 84.38 |
| Gel Electrophoresis (MC) | 37.50 | 90.00 |
| Gel Electrophoresis (SA) | 1.43 | 46.25 |
| PCR (MC) | 22.00 | 92.00 |
| PCR (SA) | 0.00 | 70.63 |
| Average | 16.91 | 77.18 |

## APPENDIX CV.

**Table 5: DNA STRUCTURE AND REPLICATION PRE AND POST-TEST**

| Question # | Pre | Boys | Girls | Post | Boys | Girls |
|---|---|---|---|---|---|---|
| | T=20 | T=20 | T=20 | T=20 | T=20 | T=20 |
| 1 | 0 | 0 | 0 | 18 | 7 | 11 |
| 2 | 1 | 0 | 1 | 17 | 8 | 9 |
| 3 | 7 | 4 | 3 | 20 | 8 | 12 |
| 4 | 8 | 2 | 6 | 19 | 8 | 11 |
| 5 | 12 | 6 | 6 | 16 | 7 | 9 |
| 6 | 1 | 1 | 0 | 20 | 8 | 12 |
| 7 | 2 | 1 | 1 | 15 | 7 | 8 |

## APPENDIX CVI.

### Table 6: BACTERIAL TRANSFORMATION PRE AND POST-TEST

| Question # | Pretest | Post-test | Difference |
|---|---|---|---|
| | T=20 | T=20 | |
| 1 | 5 | 18 | 13 |
| 2 | 4 | 19 | 15 |
| 3 | 3 | 15 | 12 |
| 4 | 6 | 17 | 11 |
| 5 | 4 | 20 | 16 |
| 6 | 2 | 14 | 12 |
| 7 | 7 | 17 | 10 |
| 8 | 4 | 15 | 11 |
| Sum | 35 | 135 | 100 |
| Ave | 4.375 | 16.875 | 12.5 |

## APPENDIX CVII.

### Table 7: GEL ELECTROPHORESIS PRE AND POST-TEST

| Question # | PreTest | PostTest | Difference |
|---|---|---|---|
| | T=20 | T=20 | |
| 1 | 9 | 19 | 10 |
| 2 | 10 | 19 | 9 |
| 3 | 9 | 17 | 8 |
| 4 | 4 | 16 | 12 |
| 5 | 8 | 18 | 10 |
| 6 | 5 | 19 | 14 |
| Sum | 45 | 108 | 63 |
| Ave | 2.25 | 5.4 | 3.15 |

## APPENDIX CVIII.

### Table 8: POLYMERASE CHAIN REACTION PRE AND POST-TEST

| Question # | Pretest | Post-test | Difference |
|---|---|---|---|
| | T=20 | T=20 | |
| 1 | 4 | 20 | 16 |
| 2 | 8 | 19 | 11 |
| 3 | 3 | 20 | 17 |
| 4 | 6 | 13 | 7 |
| 5 | 1 | 20 | 19 |
| SUM | 22 | 92 | 70 |
| AVE | 4.4 | 18.4 | 14 |

## APPENDIX CIX.

### Table 9: DEVELOPMENTAL BIOLOGY PRE AND POST-TEST

| Question # | Pre | Boys | Girls | Post | Boys | Girls | Difference |
|---|---|---|---|---|---|---|---|
| 1 | 4 | 1 | 3 | 5 | 1 | 4 | 1 |
| 2 | 0 | 0 | 0 | 7 | 2 | 5 | 7 |
| 3 | 1 | 1 | 0 | 12 | 4 | 8 | 11 |
| 4 | 1 | 1 | 0 | 5 | 3 | 2 | 4 |
| 5 | 0 | 0 | 0 | 18 | 7 | 11 | 18 |
| 6 | 0 | 0 | 0 | 19 | 11 | 8 | 19 |
| 7 | 6 | 0 | 6 | 19 | 8 | 11 | 13 |
| 8 | 2 | 0 | 2 | 19 | 7 | 12 | 17 |
| 9 | 0 | 0 | 0 | 15 | 8 | 7 | 15 |
| 10 | 2 | 1 | 1 | 15 | 8 | 7 | 13 |
| 11 | 4 | 3 | 1 | 15 | 6 | 9 | 11 |
| 12 | 1 | 1 | 0 | 12 | 7 | 5 | 11 |

## APPENDIX CX.

**Table 10: STUDENT'S GENETICS AND DEVELOPMENTAL BIOLOGY SURVEY**

| Question | Percentages of Student Responses | | | |
|---|---|---|---|---|
| | Excellent | Very Good | Satisfactory | Fairly Good |
| 1. How would you rate the laboratory experiences in this course in comparison to other science courses? | | | | |
| a. Zebrafish Development | 11.10 | 50.00 | 16.70 | 22.20 |
| b. E. coli Transformation | 44.40 | 55.60 | | |
| c. Gel Electrophoresis | 27.80 | 55.60 | 11.10 | 5.60 |
| d. All you have ALU | 72.20 | 22.20 | | 5.60 |
| 2. How well did the laboratory work relate to the ideas discussed in class? | 16.70 | 77.80 | 5.60 | |
| 3. How would you rate the classroom simulations of laboratory techniques in terms of helping you to understand biotechnology concepts taught in this course? | | | | |
| a. Breakfast DNA--> protein | 55.60 | 16.70 | 22.20 | 5.60 |
| b. Manipulating DNA Replicaiton | 33.30 | 66.70 | | |
| 4. How would you rate the Medical Technology and Genetic Testing Field Trip to MSU in terms of interest and usefulness to the study of biology? | 72.20 | 22.20 | 5.60 | |
| 5. How well did the essays relate to the ideas discussed in class? | 11.10 | 38.90 | 44.40 | 5.60 |
| 6. How do you rate the way you were graded on labs, and tests in this course? | 44.40 | 44.40 | 11.10 | |
| 7. How would you rate the laboratory classroom environment (how well students know, help and support of one another). | 33.3 | 44.4 | 22.2 | |
| 8. How would you rate the interpersonal behavior of the teacher? | 44.40 | 33.30 | | |

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