This is to certify that the

thesis entitled

A High School Molecular Biology Unit for
Honors Introductory Biology Students in
A Constructivist Classroom

presented by

David A. Devore

has been accepted towards fulfillment
of the requirements for

Masters degree in Biological Science

Major professor

Date 21 July 1994

A HIGH SCHOOL MOLECULAR BIOLOGY UNIT FOR HONORS INTRODUCTORY BIOLOGY STUDENTS IN A CONSTRUCTIVIST CLASSROOM

By

David Alan Devore

A THESIS

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

MASTER OF SCIENCE

College of Natural Science

1994

ABSTRACT

HIGH SCHOOL MOLECULAR BIOLOGY UNIT
FOR
HONORS INTRODUCTORY BIOLOGY STUDENTS
IN
A CONSTRUCTIVIST CLASSROOM

By

David Alan Devore

The purpose of the molecular biology unit was to teach basic DNA science to ninth and tenth grade honors biology students. The unit introduced DNA structure, an historical overview of DNA research, DNA replication, translation, transcription, recombinant DNA technology, and the ethical implications of such technology.

The unit was built around four laboratory activities, four modeling exercises and one class project. The instruction sought to incorporate the elements of constructivist philosophy. An additional goal of the unit was to promote student understanding of metalearning and metaknowledge.

A comparison of pre-test and post-test scores, evaluation of student journal entries, student-prepared concept maps, Vee-heuristic diagrams, entry/exit polls, and cooperative learning group reports showed that student learning was enhanced by the tactics utilized. The students were enthusiastic learners who constructed their own knowledge of molecular biology.

I dedicate this thesis to

my wife JUDY

and my son DREW

whose understanding, patience,

encouragement, and boundless love,

were my inspiration

ACKNOWLEDGEMENTS

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

Dr. Merle Heidemann

Dr. Wendy Champness

Dr. Clarence Suelter

Dr. Howard Hagerman

Dr. Marty Hetherington

Mr. Bruce Buysse

Dr. Denise Davis

Miss Kelly Cook

Miss Lisa Hauptman

Miss Karen Lorenz

TABLE OF CONTENTS

INTRODUCTION

Literature Review of the Scientific Problem

The scientific principles explained and demonstrated in this unit are (1) our current knowledge of deoxyribonucleic acid rests on accumulated experimental evidence; (2) the molecular structure and organization of DNA and RNA; (3) DNA replication; (4) transcription and translation; (5) gene mutation; (6) regulation of gene activity in prokaryotes; (7) recombinant DNA; and (8) bioethical implications of biotechnology.

David A. Micklos and Greg A. Freyer (1990) present eight questions they consider central to the development of DNA science, the first six items mentioned in the introductory paragraph. Their first question is how to account for the diversity or similarity of species. Linnaeus, in the seventeenth century, attempted to address this question with the development of systematics, the science of classification by structural similarity. Religious scholars, exemplified by Bishop James Usher, addressed the same question through a study of Biblical literature. The modern explanation of this question was derived by Alfred Wallace and Charles Darwin who posed the theory of evolution based on natural selection which was explained in the epic book On the Origin of Species in

1859. This theory described how heredity together with environmental forces molds large populations of organisms. Darwin's work was influenced by the work of James Hutton in his work Theory of the Earth, and by the publication of Principles of Geology by Charles Lyell (Micklos and Freyer 1990).

Their second question is "how are traits passed from one generation to the next?" Gregor Mendel demonstrated that individual traits are inherited in a predictable manner best explained by a "particulate" theory of inheritance. He formulated two principles to account for this particulate inheritance, the principle of segregation and the principle of independent assortment. Mendel also hypothesized that hereditary factors occur in dominant and recessive forms. The work of Mendel was corroborated and reinterpreted in 1900 by Carl Correns and Hugo de Vries (Micklos and Freyer 1990; Rasmussen and Matheson 1990; Mader 1990).

The third question posed by Micklos and Freyer is "where are the genes located?" Walter Sutton analyzed chromosome movements during meiosis in Brachystola, forming a chromosomal theory of heredity. Sutton demonstrated that meiosis in grasshoppers exactly paralleled the principle of segregation proposed by Mendel. Nettie Stevens and Edmund Wilson demonstrated in 1905 that sex is determined by individual chromosomes thus providing direct evidence to support Sutton's work. In 1910 Thomas H. Morgan's work with the mutant white-eyed fruit fly, Drosophila melanogaster proved that genes are

carried on chromosomes. His group also demonstrated the existence of sex-linkage. Alfred Sturtevant utilized the frequency of crossing over of traits to calculate gene distances and by 1920 developed the first gene maps of chromosomes in Drosophila. Another member of Morgan's group, Hermann Muller, demonstrated that X-rays increase mutation rates 1,500 times (Micklos and Fryer 1990; Rasmussen and Matheson 1990; Mader 1990).

"What is the job of the gene?" is the next question in the development of DNA science proposed by Micklos and Freyer. Sir Archibald Garrod proposed, in 1908, that some diseases in humans are "inborn errors of metabolism" caused by a defective gene inherited at birth. However, no suitable model for the study of metabolism was available to address this issue. In 1941, George Beadle and Edward Tatum introduced Neurospora as a genetic model in which it was possible to study metabolism. This mold could thrive on a minimal medium containing sucrose, inorganic salts, and the vitamin biotin. Beadle and Tatum theorized that the mold possessed enzymes which converted the minimal nutrients (plus water and oxygen) into all the complex molecules necessary for life. They irradiated Neurospora, allowed it to produce spores, and found that it would only grow on a medium to which vitamin B_6 had been added. Other mutants required other vitamins and amino acids for growth. Beadle and Tatum found that each mutant strain lacked a different enzyme needed at different points along the arginine synthesis pathway. Their work introduced the "one gene, one

enzyme" hypothesis to DNA science. The work of Linus Pauling and Harvey Itano with the hemoglobin molecule of persons with sickle-cell anemia showed that the concept could be broadened to "one-gene, one polypeptide" (Micklos and Freyer 1990; Mader 1990).

The fourth question is "what molecule is the genetic material?" In 1869, Johann Meischer isolated "nuclein" from white blood cells taken from the pus of wounds. He characterized it as rich in phosphorous but having no sulfur, properties that distinguish it from protein. Further studies characterized "nuclein" as acidic and it was named nucleic acid. By 1920 two different nucleic acids were recognized by virtue of their sugar composition, ribonucleic acid and deoxyribonucleic acid (Mader 1990; Micklos and Freer 1990) The English microbiologist Frederick Griffiths provided, in 1928, a model for answering this question. In a series of experiments with Diplococcus pneumoniae Griffiths was able to demonstrate a "transforming principle" between the virulent and non-virulent strains of this bacteria in living mice. In 1933, Oswald Avery achieved transformation outside the body of a living mouse . Colin McLeod showed that the transforming principle was not the polysaccharide coat of the bacteria. In 1944, Avery, McLeod, and Maclyn McCarty purified the transforming principle and subjected it to extensive chemical analysis, electrophoresis, ultracentrifuge purification, and enzyme testing, proving that the transforming molecule was DNA. Their interpretation was that the gene is composed of

DNA (Stryer 1988; Micklos and Freyer 1990; Mader 1990).

The answer to the question of "what molecule carries genetic information?" was further refined by the work of Max Delbruck and Salvador Luria at Cold Spring Harbor Labs, who in 1945 provided the bacteriophage as a new model for investigating how genetic material was transferred to host bacterial cells (Judson 1979). Alfred Hershey and Martha Chase performed their famous "blender experiment" in 1952. They utilized the unique properties of the phage in which an outer layer of protein surrounds an inner core of DNA. They hypothesized that if they could label both the DNA and the protein of phages they could then follow the DNA and protein through the lytic cycle. Their results showed that the daughters of the DNA-tagged phages were radioactive, but the daughters of the protein-tagged phages were not. Hershey and Chase concluded that the instructions for producing new phages were carried by DNA not protein. (Stryer 1988; Mader 1990; Rasmussen and Matheson 1990).

"What is the structure of the DNA molecule?" is the fifth question posed by Micklos and Freyer. They consider it "surely the most important biological discovery of the 20th century. In 1950 Erwin Chargoff determined that the ratio of the nucleotide bases purine and pyrimidine is always 1:1 in a variety of organisms, but that the nucleotide base composition of DNA differs from species to species. This provided evidence for the constancy required of a molecule of heredity, and also provided evidence that DNA has the required

variability as well, helping to refute the tetranucleotide hypothesis (Mader 1990). In 1951, Linus Pauling and R.B. Corey obtained precise atomic measurements of the DNA alpha-helix using ray crystallography. In 1953, Maurice Wilkins and Rosalind Franklin obtained sharp X-ray diffraction photographs of DNA showing a helical molecule with a repeat of 3.4 nm and a width of 2 nm. (Judson 1979; Stryer 1988; Micklos and Freyer 1990; Mader 1990; Kaplan 1983).

Watson and Crick used paper and metal rods to model how DNA subunits could fit into a structure that conformed to known biochemical data, and the laws of physical chemistry. They proposed a molecule composed of two antiparallel alpha-helices resembling a gently twisted ladder. The rails of the ladder run in opposite directions containing alternating units of deoxyribose sugar and phosphate. The planar nucleotides stack tightly on top on one another forming the rungs of the ladder. Each rung is composed of a pair of nucleotides held together by weak hydrogen bonds. There are ten base pairs per turn of the helix with 0.34 nm between adjacent base pairs. The overall helix therefore repeats in about 3.4 nm. In agreement with Chargoff's rules adenine always pairs with thymine and cytosine always pairs with guanine. Purines must always bond with pyrimidines if the molecule is to have the 2nm width dictated by its X-ray diffraction pattern. (Micklos and Freyer 1990; Stryer 1988)

The seventh question proposed by Micklos and Freyer is "how does DNA structure describe replication?" Watson and

Crick proposed that replication was accomplished by breaking the hydrogen bonds between nucleotides allowing the molecule to unzip. Each complementary half could serve as a template for the replication. Matthew Messelson and Frank Stahl provided evidence to support this hypothesis of semiconservative replication in 1958 (Mader 1990; Stryer 1988).

The final question posed by Micklos and Freyer is "how does DNA delineate protein synthesis?" In 1957 Francis Crick and George Gamov proposed the intellectual framework called the "central dogma." This stated that DNA is transcribed into messenger RNA which in turn is translated into protein. Further, it described the DNA sequence and the protein sequence as colinear indicating that genetic information is encoded in a linear fashion along the DNA molecule (Micklos and Freyer 1990; Mader 1990; Stryer 1988). Marshall Nirenberg and Servero Ochoa discovered that specific nucleotide sequences in groups of three, called codons, determine each of the twenty amino acids (Rasmussen and Matheson 1990). The central dogma suggests that the sequence of nucleotides in DNA and RNA must direct the primary structure of a protein. In other words the sequence of the bases in DNA determine the sequence of bases in RNA, which determines the amino acid sequence of the protein. Nucleotide base units in sets of three correlate with a given amino acid. In 1961, Marshall Nirenberg and J. Heinrich Matheii determined that the triplet code for phenylalanine was UUU. Philip Leder later designed

a system that translated only three nucleotides at a time and in this way it was possible to assign an amino acid to each of the RNA codons (Mader 1990) Several important properties of the genetic code are (1) the code is degenerate with most amino acids having more than one codon; (2) the code is definite with each triplet codon having only one meaning; (3) the code has start and stop signals (there is only one start signal but three stop signals); and (4) the code is used universally by living organisms. It has been estimated that the human chromosome contains approximately 140 million base pairs. Since any one of the four possible nucleotides can be present at a given position, the total possible number of nucleotide sequences is 4 X 140 X 10^6 or 500 million possible arrangements (Mader 1990; Arms and Camp 1991).

Transcription means making an RNA copy of a portion of DNA. In most instances it is messenger RNA that is being produced. mRNA is so named because it carries a message from the nucleus to the ribosome where protein synthesis occurs. Following transcription, the mRNA molecule has a sequence of nucleotides complementary to DNA. The RNA nucleotide uracil replaces thymine found in the DNA molecule. A portion of the DNA segment unwinds and unzips, and complementary RNA nucleotides bind with one strand of the DNA helix. When these RNA nucleotides are joined by RNA polymerase, a mRNA molecule is formed. The strand of DNA to be transcribed is termed the "sense" strand (the other is termed antisense). Antibiotics such as actinomycin D, rifamycin, and rifampicin interfere

with the process of translation. (Champe and Harvey 1987; Mader 1990).

Protein synthesis, or translation occurs at the ribosomes which are protein and ribosomal RNA (rRNA) complexes. Transfer RNA or tRNA is also involved in translation. Each ribosome is composed of two subunits, one large and one small. The subunits of the ribosome are produced separately in the nucleoli of cells, enter the cytoplasm, and remain separate until a ribosome attaches to a mRNA molecule (Mader 1990).

Transfer RNA molecules (tRNA) "know" both nucleic acid language and amino acid language and transfer RNA molecules transfer amino acids from the cytoplasm to the ribosomes. One end of the tRNA molecule contains a grouping of three nucleotide bases called the anticodon, while the other end of the molecule can bond to one of the twenty amino acids. Thus, the specific codon on a messenger RNA molecule is complementary to an anticodon on a tRNA molecule to which is attached a specific amino acid. (Mader 1990). There are 61 codons but only 30 tRNA molecules. All 61 codons are read because many tRNA molecules can bind to multiple codons (Mader 1990).

A gene is a sequence of DNA nucleotide bases and a gene mutation is a change in the sequence. Frameshift mutations occur when a single base is inserted into or deleted from a gene. Substitution of one base for another can cause a variety of things to happen. If the amino acid remains unchanged a silent mutation has occurred. If a stop codon

results from the substitution, the resulting polypeptide may be too short and nonfunctional. If the substitution results in the replacement of one amino acid with another amino acid with highly different chemical properties the function of the resulting protein may be disrupted. This is exactly the mutation that occurs in sickle-cell anemia. (Mader 1990).

The regulation of the expression of genes in prokaryotes occurs at the level of gene expression. When a gene is transcribed the enzyme RNA polymerase attaches to a special DNA sequence called a promoter. RNA polymerase then moves along the DNA joining mRNA nucleotides together. If the RNA polymerase molecule cannot attach to the promoter, RNA production and ultimately translation will not take place (Mader 1990).

Molecules which prevent the RNA polymerase from attaching to the promoter exist. They are large protein molecules called repressors. When the repressor binds to a segment of DNA called the operator, it prevents the RNA polymerase from attaching to the promoter which is adjacent to it (the operator). Repressor proteins are produced by genes called regulators (Champe and Harvey 1987; Mader 1990).

All of these elements described above work together to form a genetic operating unit called an operon. The operon model of gene regulation in prokaryotes includes the regulator, the promoter, the operator, and structural genes which are one to several genes of a metabolic pathway that are transcribed together (Mader 1990).

In 1961, Jacob and Monod presented evidence that E. coli was capable of regulating the genes necessary for lactose metabolism. E. coli normally uses glucose as an energy source. If glucose is removed as a food source and the sugar lactose substituted, the bacteria will begin to produce three enzymes needed to metabolize lactose. The production of these three enzymes is coded for by three genes (Mader 1990; Stryer 1988). The three genes are adjacent to each other and are under the control of a single promoter and a single operator. The regulator, not adjacent to the three structural genes, codes for the lac operon repressor that ordinarily binds to the operator and prevents the transcription of the three "lac" genes. When E. coli is given lactose, the lactose molecules bind to the repressor and the repressor changes shape. This change in shape prevents the repressor from binding to the operator. RNA polymerase can now carry out transcription and the three enzymes are produced. Because lactose causes the production of enzymes it is said to be an inducer of the lac operon. The unit is called an inducible operon (Mader 1990; Stryer 1988).

An understanding of basic DNA science allows scientists to utilize technology to manipulate the DNA of organisms. Bacteria are especially useful in this type of DNA technology. Plasmids are small accessory rings of DNA found in some bacteria. Plasmids are replicated when bacteria divide. They carry genes not present in bacterial chromosomes. Plasmids can be used as vectors to transfer bits of DNA or entire genes

into host cells. If a new gene is inserted into plasmids and bacteria are treated to take up the plasmids, foreign DNA will be replicated along with that of the host. This process is called DNA cloning. The introduction of a foreign gene into a plasmid requires cutting the plasmid DNA with an enzyme called a restriction enzyme. There are many different restriction enzymes but each one cuts double-stranded DNA at a particular cleavage site which is a palindrome. A palindrome is a section of DNA where the nucleotide units of each strand are identical in reverse order. This cleavage produces "sticky ends" and a gap into which foreign DNA can be inserted. DNA ligase anneals or seals the foreign DNA into the plasmid. This is a recombinant DNA molecule (Mader 1990).

Review of the Literature That Supports the Teaching Approach

> Every study or subject thus has two aspects: one for the scientist as a scientist; the other for the teacher as a teacher. These two aspects are in no sense opposed or conflicting. But neither are they immediately identical. For the scientist, the subject-matter represents simply a given body of truth to be employed in locating new problems, instituting new researches and carrying them through to a verified outcome. To him the subject-matter of science is self-contained. He refers various portions of it to each other; he connects new facts with it. He is not, as a scientist, called upon to travel outside its particular bounds; if he does it is only to get more facts of the same general sort. The problem of the teacher is a different one. As a teacher he is not concerned with adding new facts to the science he teaches; in propounding new hypotheses or verifying them. He is concerned with the subject-matter of science as representing a given stage and phase of the development of experience. His problem is that of inducing a vital and personal experiencing. Hence, what concerns him, as a teacher, is the ways in which the subject may become a part of experience; what there is in the child's present that is usable with reference to it; how such elements are to be used; how his own knowledge of the subject-matter may assist in interpreting the child's needs and doings, and determine the medium in which the child should be placed in order that his growth may be properly directed. He is concerned, not with subject-matter as such but with the subject-matter as a related factor in a total and growing experience. Thus to see it is to psychologize it.
>
> - John Dewey, "The Child and the Curriculum" (1902)

> The current state of knowledge is a moment in history, changing just as rapidly as knowledge in the past has changed, and, in many instances, more rapidly. Scientific thought, then, is not momentary; it is not static; it is a process. More specifically, it is a process of continual construction and reorganization.
>
> - Jean Piaget, "The Psychology of the Child" (1971)

The approach utilized in this unit on molecular biology is the direct modern lineal descendant of the philosophy of John Dewey and the theories of cognitive development proposed by Jean Piaget. It is often called constructivism.

Constructivism is not a theory about teaching but a theory about knowing and learning. It is based on a simple proposition: we construct our own understandings of the world in which we live. We search for the tools that will make our experiences in the world more understandable. Our experiences of the world allow us to conclude that we can get burned if we touch a burning candle, that rubber balls usually bounce while steel balls don't, that sugar is sweet, and that cubes have six sides while spheres are round. It is a common myth that knowledge is discovered, not constructed. Discovery may play a role in the production of new knowledge, but is never more than just one of the activities involved in creating new knowledge (Novak and Gowan 1993).

Each of us makes sense of the world by incorporating new experiences into what we have previously come to understand. Sometimes we encounter a new idea, object, or relationship

that does not seem to make sense to us. When we encounter such discrepant material, two courses of action are open to us. We either interpret the new experience to conform to our present set of rules and expectations for how the world works, or we generate a new set of rules that better accounts for what we are experiencing. In this way our rules and our perceptions of the world are in a constant state of tension and change as we go about shaping our understanding.

When confronted with a different experience that does not conform to our prior experience we must actively construct a different understanding that accommodates our new experience or we must ignore the new information and retain our original understanding. According to Piaget and Inhelder (1971), this occurs because knowledge comes neither from the subject, nor the object but from the unity of the two. A child playing in the ocean for the first time whose only prior experience with bodies of water was the bathtub, will be forced to change the way in which he/she thinks about water. Fosnot (1993) puts it this way: "Learning is not discovering more, but interpreting through a different scheme or structure."

As we go through different developmental periods, we are able to construct more complex understandings from the same experiences. The very young child might understand the ocean water as unpleasant tasting and burning when splashed in the eyes. The primary child might understand that the taste is salty, while the adolescent might understand the concept of salinity or be able to explain the tides. The college student

might understand how such a solution is an electrolyte and how to calculate its conductivity. Each new construction will depend upon the cognitive abilities of the person at that stage of development, the new experiences to be accounted for and the total fund of available knowledge at that time.

If we accept the proposition that we learn by constructing new understandings of relationships in our world, much of what traditionally goes on in schools must be questioned. Five characteristics of the traditional classroom are noteworthy. The typical classroom is dominated by teacher talk (Flanders, 1973). The typical teacher disseminates knowledge and then asks the students to identify and replicate the knowledge that was disseminated (Goodland, 1984). Secondly, teachers rely heavily on textbooks (Ben-Peretz, 1990). Often the information given to students is directly taken from textbooks, thus offering students only one view of the world. Third, most traditional classrooms discourage cooperation and force students to work in isolation on tasks that require very little higher-order reasoning. Further, traditional classrooms devalue student thinking. Most teacher questions are framed to discover if students know the "correct" answer rather than to discover whether the student can think through complex issues. And finally, there is often the idea in traditional classrooms that there is a fixed world that the learner must come to know. Therefore, the construction of new knowledge is not as valued as the ability to demonstrate mastery of conventionally accepted

understandings.

The consequence of these teacher-centered tactics is to encourage students to memorize material that teachers reiterate from textbooks (Goodman, 1988). The teacher then leads discussions, decides what is relevant to the students' lives, uses minimal laboratory or experiential exercises, and tends to use multiple choice tests as a means of evaluating student performance (Goodman, 1988). This type of teaching and learning revolves around the idea of performance or behavioral objectives. Performance objectives function as a means for students to master the content of material but not the concepts (Goodman, 1988). If students can be trained to repeat specific procedures or chunks of information they are said to "have learned." Typically this learning is documented by assigning grades.

Constructivism stands in contrast to this typical American approach to education. Traditionally, learning has been thought of as more of a mimetic activity where students repeat, or mime, new information (Jackson, 1986). The constructivist philosophy helps learners internalize and transform new information. Teachers can invite transformations but cannot mandate or prevent them. Thus, deep understanding rather that mimetic behavior is the goal. However, it is very difficult to describe this act of transforming ideas into broader, more comprehensive images. This is because, unlike repetition of prescribed behaviors, we cannot see either the transformed concept nor the construction

process that preceded its transformation.

To enlarge our view of the contrast between the constructivist and the traditional approach let us make a comparison of the learning environments in each type of classroom. In the traditional classroom the curriculum is presented part to whole with the major emphasis on the "basic skills." The constructivist classroom, in contrast, presents the curriculum from whole to part with the major emphasis on the "big picture." Constructivist curricula activities rely on primary sources of data and manipulative, interactive, physical materials while traditional curricular activities rely heavily on workbooks, textbooks, and ditto sheets. In the traditional classroom students are often viewed as vessels which are the repositories of information. In constructivist classrooms, students are viewed as emerging thinkers with their own theories about the world. The traditional classroom values adherence to a fixed curriculum while in the constructivist classroom the pursuit of student generated questions is highly valued. In constructivist classrooms students work in co-operative learning groups while in the traditional classroom students work alone (Brooks 1993).

Constructivist teachers also behave differently from traditional teachers. Constructivist teachers encourage and accept student autonomy and initiative rather than seeking to control student behavior. Constructivist teachers interact with their students, mediating the environment for them. They encourage students to engage in a dialogue, both with the

teacher and with one another. They frame tasks using cognitive terminology such as "analyze", "predict", "create", and "classify." Traditional teachers behave in a more didactic manner by disseminating information to students. Traditional teachers seek from students the correct answer in order to validate student learning. The constructivist teacher seeks the students' points of view in order to understand the students' present conceptions so they can be used in subsequent lessons. Further, these teachers inquire about students' understanding of concepts before sharing their own understanding of those concepts. They allow student responses to drive lessons, shift instructional strategies, and alter course content (Brooks 1993).

Assessment of student learning in the constructivist classroom is interwoven with the teaching and occurs through observations of students at work, through student projects and exhibitions, through journal entries, and through portfolios. This type of assessment is often called "authentic assessment." Assessment is authentic when it involves students in tasks that are worthwhile, significant, and meaningful (Hart,1994). In the traditional classroom student assessment is viewed as separate from the teaching process and is done almost entirely through testing (Goodman 1988).

Therefore it becomes apparent that constructivst pedagogy rests on five basic principles: (1) posing problems of emerging relevance to learners; (2) structuring learning around primary concepts; (3) seeking and valuing students'

points of view; (4) adapting curriculum to address students' suppositions; and (5) assessing student learning in the context of teaching.

Constructivist knowing and learning theory points out that students do not come to the science classroom as "blank slates" waiting to be written on. They bring with them their own conceptions of the world through which they attempt to make sense of what they read, see, and hear (Smith 1990). Many of these student conceptions fit the general world in which the students live quite nicely. Many of these conceptions are unfortunately contradictory to the scientific conceptions which underlie the science instruction to which the students are exposed (Smith 1990). The terms "naive beliefs" (McClosky 1980) and "misconceptions" (Lochhead 1988) have been used in cognitive research to discuss the idea of helping student to "change their minds" about how to interpret the world around them.

Fisher (1983, 1984) discusses five general categories of student misconceptions that affect biology instruction. The first category of misconceptions involves ideas that arise out of experiences most people share and that appear intuitively correct (on the surface) to the novice. A excellent example of this type of misconception is the belief of many secondary school students that the cells of an individual's body are different because they have different sets of genes (Hackling and Treagust, 1982, 1984).

A second set of misconceptions involves confusion between

the common meaning of a word and the scientific meaning of the same word. (Fisher, 1984) describes the confusion of the word "dominant" in genetics with "dominant" people. The use of the term "theory" in confusion with the scientific term "hypothesis" is another example.

A third group of misconceptions are those that are similar to scientific beliefs of an earlier era. Student acceptance of the theory of inheritance of acquired characteristics (Kargbo, Hobbs, and Erickson, 1980) is an obvious example.

A fourth category of misconception are thought by Fisher to be misassociations of words which he blames on "neurological hardware." The persistent confusion of students as to whether amino acids are the products or the reactants in protein synthesis is due in part to the fact that students have a stronger semantic connection between the terms "amino acids" and "translation" than between "proteins" and "translation" (Fisher, 1983).

The last category cited by Fisher is misconceptions based on alternate systems of belief. For example, difficulties would arise in the study of evolution from a creationist viewpoint.

Hawkins (1980) calls these differences between students' everyday conceptions and the scientific alternatives "critical barriers" to learning in science.

Smith (1990) sees these differences as important keys to student understanding. By specifically addressing such naive

knowledge, a larger number of students can be brought to a deeper understanding of scientific concepts. But students cannot simply be told their ideas are wrong and the alternative is right. They must be convinced. For students to change their way of thinking they must become dissatisfied with their old ways of thinking, develop an understanding of the alternative, and gradually become committed to it as they test it through application (Posner, Strike, Hewson, and Gertzog, 1982). Edward L. Smith (1990) calls this model "conceptual-change teaching."

In a learning-knowing environment designed to help students construct their own knowledge, the role that concepts play in knowledge making is central. A concept is a regularity in events or objects designated by some label (Novak and Gowan 1984). "Table" is a concept we use to designate an object with a flat top and (usually) four legs that we might use for eating. "Wind" is a concept that describes air in motion. Culture is the vehicle through which children acquire concepts and schools are institutions designed to accelerate the process of concept acquisition. The innate ability of infants and young children to recognize and label these regularities enables them to acquire speech, probably the most formidable learning task of life. Throughout early life children strive to work out regularities and apply labels. By the time children enter school they have acquired a framework of concepts and rules of language that are crucial to further progress in schooling. Children also learn methods

for organizing events or objects so that they can recognize new regularities. This allows them to recognize the labels that represent those regularities (Novak and Gowan, 1984).

Novak and Gowan (1984) believe that "concepts, and propositions composed of concepts, [are] the central elements in the structure of knowledge and the construction of meaning." One prominent learning theory that focuses on concept and propositional learning as the basis on which individuals construct their own meaning is the one proposed by David Ausuble (1963, 1968; Ausuble, Novak, and Hanesian 1978). Ausuble's theory is structured around the primary concept of "meaningful learning" as opposed to rote learning. To learn in this way student's must choose to relate new knowledge to concepts and propositions they already know. Rote learning by contrast may be incorporated into the knowledge structure of a person without interacting with what is already there.

Novak and Gowan (1984) propose that as a part of meaningful learning we must be concerned with helping students "learn how to learn." They propose that learning how to learn requires the ability to acquire metaknowledge and metalearning. Metaknowledge refers to knowledge that deals with the nature of knowledge and knowing. Metalearning refers to learning that deals with the nature of learning, or learning about learning. Novak and Gowan (1984) offer concept mapping as a tool for meaningful learning and the Vee heuristic, a diagram that relates knowing to doing, as a tool for understanding knowledge and knowledge production.

Concept maps represent meaningful relationships between objects in the form of propositions. Propositions are two or more concept labels linked by words to form a unit. A concept map is a diagram representing a set of meanings embedded in a framework of propositions.

Concept maps make clear to both student and teacher the basic ideas that they must focus on in a learning task. Because meaningful learning occurs most easily when new concepts are subsumed under more inclusive concepts, good concept maps are in a hierarchy with the more general concepts at the top. Because concept maps are an overt representation of the concepts and propositions a student holds, teacher and learner can exchange views on why a particular linkage is good and valid, or recognize missing linkages that suggest a route that learning might take. Concept maps are extremely effective in pointing out student misconceptions. A linkage between two concepts leading to a false proposition is easily noticed. Since no two students will construct exactly the same concept map given the same concepts, concept maps are helpful tools in negotiating meanings among students. Asking students to construct a group concept map requires dialogue, exchange, sharing, and compromise among the students.

In his work Taxonomy of Educational Objectives (1956) Bloom outlined six levels of objectives in education. Educators have always found it very easy to test Level I objectives calling for rote memorization. Level IV through Level VI objectives (analysis, synthesis, and evaluation) have

always been very difficult to implement. Concept mapping requires students to perform on all six levels in one composite effort making such evaluation possible (Novak and Gowan 1984).

The Vee heuristic, invented by Gowan in 1977, was first used to help students clarify the nature and purpose of laboratory work in science classes. At the point of the Vee are the events or objects to be observed. To the right are the methodological elements and to the left the conceptual elements. The left side of the Vee represents "thinking", the kinds of conceptual and theoretical ideas used in scientific inquiry. The right side of the Vee represents "doing", the methodological and procedural activities the students undertake. The Vee is an attempt to integrate the thinking side with the doing side. Gowan and Novak (1984) state the problem this way:

> In school science laboratories, students may be engrossed in making records of observations of events or objects, transforming these records into graphs, tables, or diagrams, and drawing conclusions or "knowledge claims"-often without knowing why. Rarely do students deliberately invoke relevant concepts, principles, or theories, in order to understand why specific events or objects have been chosen for observation, why they are making certain records, or certain kinds of graphs or tables, or why their conclusions from the data are often "wrong" when judged against the textbook or other authority.

The Vee helps student see that although the meaning of all knowledge derives from events or objects we observe that there is nothing in these events or objects that tells us what

the record means. This meaning must be constructed. The Vee becomes a tool for acquiring metaknowledge, that is knowledge about knowledge and how knowledge is constructed and used. Gowan's complete Vee (1984) is reproduced in Appendix G.

To create environments in which teachers and students are encouraged to think and explore is a formidable task. But to not take up the challenge is to perpetuate the ever-present behavioral approach to teaching.

Piaget (1969) wrote:

> The heartbreaking difficulty in pedagogy, as, indeed in medicine and in many other branches of knowledge that partake at the same time of art and science, is, in fact, that the best methods are also the most difficult ones: it would be impossible to employ a Socratic method without first having acquired some of Socrates' qualities, the first of which would have to be a certain respect for intelligence in the process of development.

Statement of the Problem and Rationale for Study

My thesis demonstrates the effectiveness of a constructivist pedagogy in helping students acquire new knowledge of molecular biology. I designed an instructional unit incorporating the five main principles of the constructivist pedagogy: (1) posing problems of emerging relevance to learners, (2) structuring learning around primary concepts, (3) seeking and valuing students' points of view, (4) adapting curriculum to address student's suppositions; and (5) assessing student learning in the context of teaching.

I addressed the effectiveness of student-centered teaching methods on student learning and knowledge. These student-centered teaching tactics included co-operative learning groups, laboratory experiences, learning exercises involving manipulation of materials, a student project, journal entries, concept mapping, and the use of the Vee heuristic.

I hypothesized that students taught with a constructivist approach would show growth on the mean score of an evaluative test.

The rationale for choosing such a study is closely tied to my development as a science educator. My original scientific training in undergraduate school was in the field of botany and ecology. My training in science teaching

methods was based upon the "inquiry approach", an outgrowth of the curriculum reform projects of the 1960's. For many years I taught biology using the BSCS Green Version Biology textbook as my curriculum. It was also my original vision of exemplary biology instruction.

As I progressed in my teaching career it became apparent that the inquiry approach to teaching was primarily just that, an approach to teaching. It did little to address the needs or interests of the students as learners. Indeed, I came to realize that the Green Version biology which so appealed to me as an ecologist was nearly incomprehensible to the average to superior tenth grader. Graduate studies in education which I undertook also convinced me that the inquiry method of instruction as represented in the BSCS curriculum projects did not address the increasingly large body of knowledge in the fields of learning theory and cognitive development.

At the same time, I was becoming uncomfortably aware that the field of biology was changing around me. It had been fifteen years since I had last been a biology student myself. Young colleagues joining the faculty of my school increasingly made my own obsolescence apparent to me. These bright-eyed young people were discussing a "new biology" based on concepts in cell biology, biochemistry, and molecular biology. Textbooks were changing too, and as I moved from the use of the increasingly useless-BSCS inspired texts to introductory college-level texts it was obvious that much of the content in these texts was biology that I had never studied formally. I

laughingly told one of my young colleagues that fifty percent of what I was teaching wasn't even known when I graduated from college, but it wasn't a laughing matter. It was obviously time to retire or retrain in the "new biology."

I decided to begin my retraining in a series of summer workshops for teachers at Michigan State University. As I became more comfortable with the topics of cell biology, molecular biology, and biotechnology my desire grew to incorporate these topics into my biology curriculum in a meaningful way. I saw a new vision of a biology curriculum structured around active investigation and experiential learning that would make molecular biology come alive for the students. The instructors and participants in these summer sessions shared the vision of a more experiential way of teaching molecular biology as well and the synergy was exhilarating.

The missing element was a coherent theory of learning and knowing around which to structure the molecular biology unit. I discovered this element through my involvement with a school-based teacher apprentice program of a local university. The young graduate student teachers-in-training teaching in my school's classrooms brought with them a constructivist approach to learning and knowing based on the philosophy of John Dewey, the adolescent psychology of Erik Erickson, the cognitive theories of Jean Piaget, the moral development theories of Lawrence Kohlberg and Carol Gilligan, and the belief of Joseph Novak and Bob Gowan that children can learn

how to learn. These ideas empowered me and gave me a new vision of what could happen in my classroom. Constructivism really is the way I've always known people learn, but I was unable to see my way clearly to incorporate these ideas in a coherent way in the teaching of molecular biology. Thus the impetus for the study presented in this thesis is really just one teacher's dissatisfaction with the status quo and the quest for a better way to do things. It is a good model of how we construct new understandings of the world in which we live.

Although experiential learning was an important element of other portions of the biology curriculum, it was a new element in the teaching of molecular biology. Previously the study of the structure and replication of DNA had been textbook and lecture based with no laboratory work incorporated into the curriculum. I usually allotted three days for "completion" of this topic. DNA and the process of transcription and translation were taught in a similar fashion with the addition of a twenty minute video presentation on protein synthesis. The time assigned for this study was an additional three days. Evaluation was by means of a multiple choice test administered after both topics were completed. There were no elements of constructivist philosophy in this teaching unit. The topics of gene regulation, an historical perspective on DNA science, and any consideration of bioethical implications of the emerging biotechnologies were nonexistent.

The new molecular biology curriculum did not emerge all in one piece. It was an evolutionary process that stretched over four years. As I became more familiar with molecular biology I began to slowly incorporate new laboratory activities and especially modeling activities into my teaching. The instruction became less teacher-centered and more student-centered. Thus, the old approach to teaching DNA science slowly faded away to be replaced by the model presented in this thesis. This evolutionary process, plus the four hundred percent increase in instructional time devoted to consideration of DNA science, precluded a meaningful comparison of students taught in the "old" didactic, teacher-centered classroom with those taught with the "new" constructivist approach.

The decision to expand the molecular biology portion of the biology curriculum from six instructional days to twenty eight instructional days meant that other parts of the biology curriculum had to be reduced or eliminated. I chose to eliminate a small unit on bacteriology, believing that the transformation experiment in the new unit could serve the same function as the previous instruction. The majority of the increased instructional time came at the cost of eliminating the entire unit on animal anatomy and physiology. This unit had been built around the dissection of the fetal pig or the cat (alternate years). This was the only dissection undertaken in this introductory course and it was an experience that I was reluctant to eliminate.

In sequence the molecular biology unit follows the study of populations and ecosystems, a study of basic biological chemistry, the study of cell ultrastructure, the study of bioenergetics, the study of patterns of heredity, and a consideration of human genetic disorders. It precedes the study of population genetics and evolution. Due to its positioning late in the curriculum students are adept at following laboratory procedures and using equipment. This was a major element of concern in the implementation of this unit as the laboratory skills and even the manual dexterity of the average fifteen-year-old boy are not high. The students are also comfortable with co-operative learning activities at this point in the year. The students are also familiar with keeping journals of their laboratory observations and are acquainted with the transformation and analysis of data. More specific knowledge skills required to undertake the molecular biology unit are discussed under the implementation of the unit.

The materials incorporated into the molecular biology unit came from a wide variety of sources. The idea of presenting DNA science as a series of questions in a time line of scientific progress was garnered from two sources, DNA Science: A First Course in Recombinant DNA Technology by David Micklos and Greg Freyer (1990) and A Sourcebook of Biotechnology Activities, edited by Alison Rasmussen and Robert H. Matheson III (1990). The cardboard DNA model used to build the DNA class project was adapted from Problem

Solving in Biology, Third Edition, by Eugene H. Kaplan (1983). The two exercises "DNA Structure" and "DNA Replication" were found in a kit purchased from Carolina Biological Supply Company, Burlington, N.C. The DNA isolation and spooling laboratory was adapted from A Sourcebook of Biotechnology Activities as was the modeling exercise "How Genes Makes Proteins." "Properties of DNA" was adapted from the instructions in a kit supplied by Modern Biology, Inc., Dayton, Indiana.

The laboratory "Transformation of E. coli" was written from material gathered in my own experimental work in bacterial transformation and followed standard procedures available in a wide variety of sources. "Gel Electrophoresis: Restriction Mapping of DNA Fragments" is another standard laboratory procedure. The specific procedure utilized in the restriction digest was provided by Modern Biology, Inc., the supplier of the materials. The electrophoresis apparatus was constructed following plans developed by Dr. Clarence Suelter, Michigan State University and presented in a teacher workshop "Biological Science for Teachers", summer quarter, 1989. The electrophoresis process and staining technique was also adapted from the same summer workshop. The modeling exercise "Production of a Recombinant Plasmid" was adapted from the article presented by Christie L. Jenkins in The Science Teacher, April, 1987.

IMPLEMENTATION OF UNIT

Basic Outline:

The instructional unit in molecular biology was based on Chapters 15, 16, 17, and 18 of Biology: Third Edition by Sylvia Mader (1990). The textbook reading was supplemented by three laboratory exercises, five activities involving manipulation of materials or ideas (often called "dry labs"), parallel reading in other sources, lecture notes, and video tape. The unit took 28 days to complete including one day for pretesting and one day for post test assessment.

The instructional unit was implemented at University School, Chagrin Falls, Ohio, in the spring trimester, 1992. University School is an independent country day boy's school with an enrollment of 800 students in kindergarten through grade twelve. Admission to the school is selective. Class sizes are small and the curriculum is research-oriented. The curriculum is highly student-centered, interdisciplinary and theme-based in the elementary and middle schools. The upper school is highly departmentalized and instruction is more didactic and teacher-centered although the school is in transition to a more student-centered environment.

The unit was introduced into three honors introductory biology classes with a total of 48 students. The classes were

mixed freshman and sophomore boys. None of the students had studied biology previously, although most had been exposed to the life sciences in middle school. The classes met for three forty-five minute periods per week and two ninety minute periods per week. The ninety minute time blocks were utilized for laboratory and activity-based instruction. The forty-five minute time blocks were used for direct instruction, pre-laboratory instruction, interim assessment, class discussion, and student co-operative learning group work.

A variety of instructional techniques were utilized to address the different learning modalities of the students. Visual learners were engaged through the use of overhead transparencies and video tape presentations on the structure of DNA, DNA replication, and the process of transcription and translation. Auditory learners were engaged through lecture, class discussion, and parallel reading activities on the same topics. Kinesthetic learners were engaged by the use of a variety of manipulatives including "poppit bead" models of both the structure and the replication of DNA, the construction of a paper model of the DNA sequence necessary to code for porcine proinsulin, and a paper model of the production of a recombinant plasmid. All student learning styles were engaged by laboratory exercises based on the bacterium *E. coli*. Students extracted DNA from this bacteria, and transformed *E. coli* with the plasmid pUC18. Students carried out a restriction digest and performed a gel electrophoresis experiment to determine the length of DNA

molecules.

Students had studied basic cellular chemistry in a previous unit of instruction in this same course. They had developed mastery of chemical bonding, molecular formulas, structural formulas, condensation reactions, hydrolysis reactions, redox reactions, and energy transformations. Students were able to recognize the structural formulas of macromolecules and their monomer units, including the nucleic acids. Students had also mastered basic genetics and had demonstrated a good conceptual understanding of cell ultrastructure and function.

The instructional unit was presented in an historical context. This allowed students to gain an appreciation of how scientific knowledge is acquired. They understood that our current knowledge of the structure and role of DNA is based on accumulated experimental evidence.

Student learning was monitored through the use of student-prepared concept maps, entry and exit polls, interim quizzes, reports from cooperative learning groups, periodic review of the laboratory notebook of each student, and pre testing and post testing.

A clinical interview with three students was conducted prior to the introduction of the unit to assess the pre-existing knowledge of the students. A second interview with the same students was conducted at the conclusion of the unit to assess how naive knowledge of the students had been changed and to assess their perception of the value of the

instructional unit.

The first week (5 days) of instruction was an introduction to the history of the major discoveries that have lead to our current knowledge of DNA. In the laboratory students explored the chemical and physical nature of DNA in two experiments: Extraction and Spooling of DNA and Properties of DNA. The second week of the unit introduced students to the Hershey and Chase experiment through a video presentation. Two modeling activities: DNA Structure and DNA Replication allowed students to get "hands-on" experience with the molecule. The second week of instruction closed with a consideration of Beadle and Tatum's experiment with Neurospora.

The third week of the unit focused on the central dogma of molecular biology. Students viewed a video presentation, "The Mechanism of Protein Synthesis." Two modeling exercises, How Genes Make Proteins and "A Class Project, a DNA Model of Proinsulin were utilized to reinforce the concepts of transcription and translation. The concept of recombinant DNA was also introduced.

The fourth week of the unit concentrated on biotechnology activities. Students completed a modeling exercise, A Paper Model of the Production of a Recombinant Plasmid. The major activity of the week was the completion of the laboratory Bacterial Transformation of E. coli with pUC18.

In the fourth week students completed the laboratory on Gel Electrophoresis: Restriction Mapping of DNA Fragments.

The unit concluded with a consideration of the ethical implications of biotechnology building on ethical decision making skills the students had acquired in a previous unit of instruction.

Appendix A is a detailed daily summary of the unit activities. Appendix B contains the text of the laboratory activities utilized in the unit. Appendix C contains the text of the modeling activities of the instructional unit.

Audio-visual Aids Used in the Unit

The chemical aspects of biology are especially difficult for high school students to conceptualize. Many ninth and tenth graders, the age of the boys to whom this unit was presented, are still at the developmental stage of concrete operations (Piaget, 1971). Those boys who have attained their formal operations have a less difficult time conceptualizing molecular organization and activity. Learning at all levels of thinking and conceptualizing is aided by well-designed, colorful overhead transparencies.

Publishers of science textbooks are very cognizant of the need to provide such teacher resources. Wm. C. Brown, the publisher of the textbook on which this unit is based Biology: Third Edition by Sylvia Mader (1990) provided 17 transparency overlays that supplement the text presentation of molecular biology. I used these in my classroom presentations on the structure of DNA, DNA replication, and the transcription-translation process.

To help students visualize the investigations that were undertaken that have led to the development of DNA science, overlays of the work of T. H. Morgan, Beadle and Tatum's Neurospora experiment, Griffith's transformation experiment with Pneumococcus, the Hershey-Chase blender experiment, and the Meselson-Stahl heavy/light nitrogen experiment were

utilized. These overlays were gathered from a variety of sources and are reproduced in Appendix D, 1 through 6

Student mastery of the concept of the central dogma of molecular biology as articulated by Francis Crick was aided through an animated film on the transcription-translation process. This twenty minute video, produced by the Biochemical Society in London, England, is clear, concise, and entertaining. Student reaction to the use of this video was unusually positive for it allowed the students to view transcription-translation as a dynamic process.

I also showed students a video tape segment re-creating the Hershey-Chase experiment produced by the Biochemical Society, London. The students found it to be confusing and much too difficult. I used an overlay and a brief discussion to deal with the confusion caused by this video. I do not plan to use it again.

The history of DNA time line was presented as a guided lecture (students were given an incomplete set of lecture notes), intermittently over several days. To help students organize this material they were given a handout of this lecture with the questions outlined, and the scientists listed. They completed the handout as we discussed the experimental contribution of each scientist. An overlay of the timeline was utilized in this discussion and is reproduced in Appendix D, 7 through 9.

Pedagogical Value of the Laboratory Exercises

The purpose of the four laboratory exercises and the five learning exercises involving the manipulation of materials or ideas (the "dry laboratories") was to provide a curriculum designed to engage students effectively in the learning process. High school students view molecular biology as bewildering, arcane, and beyond their ability to comprehend. Students also persist in the belief that the study of DNA can only be undertaken at an advanced level by highly trained "scientists." In the past I have found that my students resist engagement in the topic of molecular biology beyond a very naive conceptualization. By utilizing simple, homemade, materials that are a part of the everyday world of the students, I hoped to demystify the topic. My goal was to help my students construct their own knowledge of DNA by making a connection to the real world. Further, I wanted them to view molecular biology as something that anyone can understand and not as something that is the exclusive purview of an elite few.

The objective of the first experiment, The Extraction and Spooling of DNA, was to allow students to actually see and touch DNA. This was a new experience for all of them. Although the students were excited by the experience of spooling the long threads of DNA onto the stirring rod, they

were also somewhat disappointed. The result, the white gummy mass of DNA on the stirring rod, didn't really seem like anything very special to them. Several students commented that when they viewed the DNA under the microscope they couldn't see the double helix or the "letters". This was exactly the type of previously acquired naive knowledge (misconception) that I was hoping to confront in this unit. This so-called "snot-on-a-stick" experiment allowed the students to begin to experience DNA as a physical reality, and to begin to comprehend its scale. I assessed student learning through the summary of observations and conclusions each student wrote in his laboratory journal.

The second experiment, The Properties of DNA, was designed to build on the curiosity about the physical nature of DNA engendered by the first experiment. This exercise permitted the students to explore the physical nature of the DNA molecule. The laboratory introduced the concepts of denaturing DNA and then cutting DNA with enzymes. This laboratory, together with the first one, provided the students with a conceptual model of DNA as a part of the real world rather than as a two-dimensional, multi-colored illustration in a textbook. Evaluation of student learning took place through laboratory journals.

The first modeling exercise, DNA Structure, was undertaken after students had been introduced to the experimental evidence behind the Watson-Crick model, had read about the structure of DNA, and had discussed the

structure of the molecule. This exercise utilized a modeling kit ("DNA Simulation BioKit") obtained from Carolina Biological Supply Company. This modeling kit provided an opportunity for students to master the structure of DNA in three dimensional form. The kit utilizes "poppit beads" with a prong connector on one end of the round bead and five holes evenly spaced around the remaining surface of the bead. The beads come in six colors to represent deoxyribose sugar, phosphate, and the four nitrogenous bases. The bead representing the sugar deoxyribose has further designations for the 3′, 1′, and 5′ positions of the carbon atoms. Students are instructed to prepare sixty individual nucleotide units. They are then instructed to prepare a single polynucleotide chain and are directed to build a complimentary antiparallel chain. Clear plastic connectors supplied with the kit represent hydrogen bonds between purine and pyrimidines. Students then twist the model to illustrate the spiral of the DNA molecule.

This model provides an opportunity for students whose dominant learning modality is kinesthetic to master the concepts of the nucleotide, complimentary base pairing, the 3′,5′ orientation of the deoxyribose sugar, and the antiparallel nature of the complimentary strands. As the laboratory pairs constructed this DNA model, I met with each group and informally asked a series of questions to assess their level of understanding. Each student was also asked to write in his laboratory notebook a summary of what he had

learned from the exercise. My assessment of the student groups lead me to realize that the model, through its design, was promoting two misconceptions about the structure of the DNA molecule. The first was that all the component molecules of a DNA nucleotide are the same size and shape. This conclusion was drawn because the model utilized the same size and shape of beads to represent all the component units of the nucleotide. Secondly, the model did not distinguish the differences in hydrogen bonding between adenine and thymine as a pair and guanine and cytosine as a pair. It utilized the same "dog-bone- shaped" connector for both bonds. Therefore students were unable to visualize the reason for complimentary base pairing. I addressed these misrepresentations with each group, and by leading a general class discussion on the nature and shortcomings of any model of reality. This was a valuable teaching opportunity I had not anticipated.

The second modeling exercise, DNA Replication, also utilized the DNA simulation kit. The students assembled a simulated DNA sequence 30 nucleotide units in length, then separated the two complementary antiparallel strands in small steps and carried out the replication process. This allowed students to visualize the concepts of the leading strand, the lagging strand, and the idea that the lagging strand must replicate in short, discontinuous segments (Okazaki fragments) to keep pace with the separating template strands. The concept that DNA replication occurs in a 5′ to 3′ direction on a 3′ to 5′ template was clearly illustrated. By allowing

students to build their own model of DNA replication, a process that formerly had been extremely confusing to understand, had been rendered intelligible. The model reinforced the text readings and class discussion and provided a new opportunity for students to construct their own knowledge. Class discussion and an analysis of laboratory notebook entries concerning this exercise clearly showed that the students had mastered the concept of replication.

The third modeling exercise, "How Genes Make Proteins," taken from A Sourcebook of Biotechnology Activities published by the National Association of Biology Teachers (1990) was done after students read about protein synthesis. Students acted out the process of transcription and translation using the classroom itself as a model of the cell, with the walls, floors, and ceiling representing the plasma membrane. An area bounded by chairs spaced at intervals represented the nucleus and nuclear membrane with pores. Cards representing DNA sequences and their complementary mRNA molecules were given to two groups of students. These students carried out "transcription" in the nucleus of the cell. The students representing the "mRNA" molecule left the "nucleus" and entered the "cytoplasm" of the cell. Another group of students were given tRNA cards and amino acid cards. After linking up the "tRNA's" with their proper "amino acids" (only seven amino acids were represented) the pairs of students representing the "tRNA-amino acid" linkage used the concept of complementary base pairing to link up with the students

representing the "mRNA" molecules to complete translation.

The students also watched a video presentation of the protein synthesis. My goal was to reinforce the concepts previously presented by allowing students to actively engage in modeling of the transcription and translation process. I hoped to help students construct a new understanding of the triplet code, to recognize the significance of complementary base pairing, to master the concepts codon and anticodon, and to understand the roles of mRNA and tRNA. This activity also allowed me to extend a previous class activity, "A Cell Metaphor". Completed several months before the molecular biology unit, this model had allowed students to construct a classroom-sized cell. Cellular organelles were represented by common objects but were in scale and proper numerical relationship to each other. Student journal entries in the form of answers to the questions posed after completing this exercise showed a mastery of protein synthesis. However, student opinion was sharply divided on the value of the activity in helping them achieve mastery. Those students who found the model unhelpful were so vocal in their objections that I probably will not use this activity again.

The fourth modeling exercise was the building of a cardboard model of a sequence of DNA which could code for a small protein molecule, porcine proinsulin (Stryer, 1988). This activity also engaged the students in a cooperative enterprise, the outcome of which was dependent on the active participation of every class member. Students worked in their

designated cooperative learning groups of four students. The class was presented with the primary structure of the proinsulin molecule. The groups were given duplicated pages of paper models of the component units of the DNA molecule. The different monomer units had been placed on different colors of paper. Students were instructed to paste these pages onto light cardboard and to cut out the individual units. Each group was then told to work backwards through the transcription and translation process to determine the DNA sequence that would code for the portion of the polypeptide chain that was their responsibility. The "left-hand" side of the DNA molecule was arbitrarily chosen to be the sense strand of the molecule. The groups were given four days to produce their contribution to the model. On construction day, the entire model, 252 base pairs in length, and over 15 actual feet in length was assembled by the groups. The students hung this spectacular representation of the DNA needed to code for a small protein from the classroom ceiling where it remained through out the rest of the unit on molecular biology. The students were pleased with the outcome of this activity and brought in other students to see what they had built. I was pleased with the progress the students had made since September (this activity occurred in February) in organizing and accomplishing group tasks. No further student assessment was attempted.

On the same day that the class constructed the model of the proinsulin DNA, they also completed modeling activity V.

This paper model of a recombinant plasmid followed a class discussion of recombinant DNA technology. The progress of the class discussion on restriction enzymes, sticky ends, palindromes, plasmids, and cloning convinced me of the need to use this visual and kinesthetic activity. The activity allowed students to construct their own mental image of a plasmid, how it might be cut by a restriction enzyme, how different restriction enzymes might cut the plasmid differently, and how the cut plasmid might be annealed again. It was crucial that students master these concepts as a prerequisite the next two laboratory activities. This activity worked very well. A summary of the activity, in the form of answers to questions, which each student wrote in his laboratory notebook, showed that the students had mastered the concepts necessary to complete the laboratory on the transformation of the bacteria E. coli.

The laboratory on the transformation of the bacteria E. coli was the most difficult laboratory procedure the students had attempted all year. I spent a forty five minute period on pre-laboratory instructions and another forty five minute period having the students practice the techniques necessary to make this laboratory experiment, and the following laboratory, a success. This was the first time the students had utilized microliter quantities of substances or used micropipette apparatus. It was also their first exposure to basic bacteriological techniques. This was time well spent as the students carried out the actual laboratory procedure

with confidence and a minimum of confusion. In retrospect, the experiment would have been more intelligible if the students had seen an actual demonstration of the effects of antibiotics on bacterial growth previous to doing the transformation. I will demonstrate the effects of antibiotic sensitivity discs on bacterial growth when teaching this unit in the future.

Students were given an overnight culture of E coli to be transformed. The cells were made competent by treatment with calcium chloride. One sample of the competent cells was allowed to take up the plasmid pUC18 containing a gene for resistance to the antibiotic ampicillin (+ DNA) while the other sample was not exposed to the plasmid (- DNA). Both samples of bacteria were incubated with Luria broth and then plated onto LB agar containing ampicillin. The second day the colonies on each plate were counted. There was good growth of colonies on the "+DNA" plate and a lesser growth (although some growth) on the "- DNA" plates. This demonstrated that the E. coli bacteria which had incorporated the plasmid were now resistant to the antibiotic ampicillin.

The students then began the portion of the experiment designed to illustrate the "lac operon." Each group was given two LB plates containing ampicillin, X-gal, and IPTG, an inducer of B-galactosidase activity in the bacteria. If the bacteria switched from using galactose as an energy source to using lactose as an energy source, the X-gal in the medium would react with the enzyme B-galactosidase produced by the

bacteria to form a blue product which would "stain" the colonies. The students spread 200 microliter samples of the previously transformed E coli onto the X-gal/IPTG/amp plates. The next day the students counted the number of blue colonies present on the plates. The groups then calculated the transformation efficiency of the LB/X-gal/IPTG/AMP plates.

Although the laboratory activity was successful for most groups, the students had great difficulty transferring knowledge about what happened in the experiment to a discussion of the lac operon. I required the students to prepare the laboratory summary of this experiment utilizing the "Vee" heuristic. This was an attempt to promote student understanding of the laboratory activity by asking them to invoke relevant concepts, principles, and theories to explain why the procedure had been done. The students asked for a day to meet in co-operative groups to write a summary of the laboratory activity and to study the "lac operon" model in their reading. They also met with me individually. The results, incorporated into their laboratory journals, showed that less than half of the students had mastered the concept. A free response question on the "lac operon" incorporated into the post test elicited the same response from the students. I reluctantly concluded that the majority of ninth and tenth grade students in this honors biology group were not developmentally ready to construct this knowledge.

The last laboratory activity, Gel Electrophoresis, showed how two restriction enzymes cut DNA (in this case

lambda DNA), and how the fragments of DNA can be separated and identified using gel electrophoresis. As part of the pre-laboratory discussion, a senior student from my research class, who was using gel electrophoresis to explore the genetic distance among various races of brook trout, explained his work to the classes.

The electrophoresis was accomplished with "homemade" plastic-tray gel electrophoresis boxes constructed in a teacher workshop at Michigan State University. Although more sophisticated equipment was available I utilized this simple apparatus as a part of my goal of bringing "pieces of the students' real world" into the classroom. I used variable power supplies, borrowed from the physics teacher, as the power source. The gels were run at 30 volts.

The students prepared a restriction digest of lambda DNA using EcoR1 and BamH1 separately and in combination. These digests were mixed with glycerol, loaded into the wells of a previously prepared 1.2% agarose gel, and run against a predigested DNA sample and a running dye supplied in a materials kit which was purchased from Modern Biology, Inc. The gel was run for two hours, then stained with methylene blue and refrigerated overnight. The next day the distance from the well to the bands of dye in each lane was measured in millimeters and recorded. The pre-digested sample of phage DNA had been cut with the restriction enzyme HindIII yielding six fragments of known length. Using semi-logrithimic graph paper, the students established a plot line with the known

fragments produced by the HindIII digest. They plotted migration distance on the x axis. The size of the fragments, in base pairs, was plotted along the y axis. The DNA fragments from the EcoRI and BamHI digests were then compared to the standard plot and the size of each fragment estimated.

The students enjoyed this laboratory more than any other they attempted in the molecular biology unit. They liked to work with the equipment. They also found the visible results very satisfying and easy to comprehend. They were aware of the practical applications of this technique by having been introduced to the experimental work underway in other classes.

At the time we undertook this laboratory there was discussion in the popular press on the role of DNA in identifying criminals and the accuracy of such identification techniques. This set the stage for a discussion of DNA fingerprinting and its ethical and legal implications.

Since the completion of this unit I have been introduced to an excellent DNA fingerprinting simulation that would illustrate these concepts (1992 Woodrow Wilson Institute on Bioethics, Princeton University). In a Howard Hughes Medical Foundation biotechnology workshop at Case Western Reserve University I also learned how to extract DNA from cells of the buccal cavity, perform a polymerase chain reaction with this material, and prepare an actual DNA fingerprint which can be given to students in the form of a photograph. I will utilize this new material when I teach the unit again.

New Teaching Techniques

The innovation in this unit in molecular biology lay not so much in its individual elements but in their application in conjunction with the constuctivist theory of teaching and learning. All of the laboratory exercises and learning exercises utilized in the unit are drawn from previously existing sources.

In this unit I attempted to engage students actively in the construction of new knowledge which would replace their previously acquired naive knowledge through a more experiential and laboratory-based curriculum. These student-centered methods "enhance thinking and problem-solving skills" (Goodman,1988), encourage student-student and student-teacher interaction, promote independent thought, and help establish a link between classroom instruction and the external environment. The goal of this type of instruction is to stimulate students intellectually to learn from and through experience in order to avoid becoming passive recipients of knowledge (Dewey,1964).

A further innovation was to have students test this knowledge through operation. Dewey claims that "while the student with a proper 'project' is intellectually alive, he is also overtly active; he applies, he constructs, he expresses himself in new ways" (Dewey 1964). This permits a connection

between past experiences, new material and knowledge, and external interest that needs to exist in order to maximize knowledge learning.

All of the laboratory activities, learning exercises, and student projects in this molecular biology unit were chosen to reflect and implement this philosophy of teaching and learning based largely on the work of John Dewey and Jean Piaget as outlined previously. Biochemistry in general, and molecular biology in particular usually is not explicable in ordinary, everyday terms. This separates students from the subject and makes it appear that access to this knowledge is open only to the most highly trained individuals in our society. This unit was an attempt to overcome these difficulties and promote student understanding that science is for everyone.

The pedagogy utilized new and innovative theories of knowing and learning based on the research of Novak and Gowan. Part of the goal of the unit was to help students "learn how to learn." The acquisition of metaknowledge and the enhancement of metalearning were a key component of the curriculum of the molecular biology unit. Preparation of concept maps has been shown to be a valuable tool in aiding students to acquire self-knowledge of how they best learn. To this end, students constructed concept maps of the transcription-translation process. They also constructed concept maps of the operon theory of the control of protein synthesis. Examples of these student concept maps are included in Appendix G.

The students were also introduced to the "Vee heurestic". This tool of metaknowledge developed by Novak and Gowan is extremely useful in helping students assess what they have learned from laboratory activities. I required the students to use the Vee as the summary of the transformation of E. coli experiment. I encouraged its use in summarizing other laboratory activities as well. An example of a student's Vee-heuristic diagram is included in Appendix G.

Daily student mastery of the unit was assessed through the use of entry and exit polls. Some days students were asked, before dismissal, to write down one thing they had learned from class that day. On other days students were asked to write down one thing from that day's class work that they were still confused about. This allowed me to address student learning on a daily basis. I was able to adjust the curriculum to meet the needs of the students. Entry polls were used to assess assignments given as homework. Students were asked to prepare ahead of time a slip of paper on which was written one or two things they had been unable to understand from the previous night's assignment. This was usually text reading or laboratory summary writing. The students handed these slips to me as they entered class (their admission ticket I told them). I could quickly leaf through the entry polls to assess the level of understanding of the class and determine where to begin the day's lesson.

The use of entry and exit polls allowed me to assess whether or not the level of the curriculum was appropriate to

the level of conceptual development of the students. Within the first two days I discovered that the textbook Biology: Third Edition by Sylvia Mader was something of an albatross in the opinion of the students. This is a widely used introductory college text with a reading level of grade 11.5. I had chosen the text because I believed it to be at an appropriate level for these honors biology classes in a selective preparatory school. Although they had used the book all year, the students found the chapters on molecular biology, Chapters 15, 16, 17, and 18, to be especially content dense. Further, they had difficulty following the organization of the discussion of the topics under consideration. To circumvent this problem, I introduced parallel readings from another textbook A Journey Into Life, 2nd Edition by Arms and Camp (1991). This strategy worked well as indicated by a decrease in the confusion noted on the entry polls. The textbook was only one learning resource of many in the unit. I believe that the goals of the unit could have been successfully achieved without the use of any textbook.

One laboratory that I had originally planned to incorporate into this unit was eliminated. The laboratory involved the extraction of the plasmid pUC18 from transformed E coli. In my own research in constructing the teaching unit I could not consistently extract the plasmid utilizing a millipore filtration apparatus. Because student learning at this level is often frustrated by negative results I did not

incorporate the laboratory into the unit. Time considerations eliminated any other possible plasmid extraction method.

My original research for this unit involved using the plasmid pUC18 with the "lux" gene for bioluminescence. This plasmid transforms E. coli to be bioluminescent when provided with an oxidizable substrate such as dodecyl alcohol. Attempts to complete this laboratory with a small test group of students prior to the introduction of the entire unit were unsuccessful. In addition, the alcohol substrate needed to produce the bioluminescence was an extreme chemical irritant. For these reasons I chose to complete the bacterial transformation using X-gal as a visible result of the process.

EVALUATION

Pre-Test and Post-Test Results

Student growth in mastery of the concepts presented in the molecular biology unit was measured by a test administered to the students before the unit was taught and again at the end of the unit. The pre-test consisted of 57 multiple choice questions evenly distributed over six of the seven content areas of the unit. I chose not to test over the bioethics material. Utilizing Bloom's Taxonomy of the Cognitive Domain to rank the questions, thirty six of the questions were knowledge questions, sixteen of the questions were comprehension questions, four of the questions were application questions, and one question was an analysis question (see Appendix F, Post-test Item analysis).

The test was administered, without advance notice, to 48 students, mixed ninth and tenth graders, in three different sections of introductory biology. None of the students previously had been enrolled in a biology course. The students had no common background in science. Thirty of the students were from the University School middle school while the remaining eighteen were enrolled out of sixteen different public and private schools.

The students were told that the test would not be used to

determine their grade on the molecular biology unit, but that they should make their best effort to answer every question. The students seemed to comply with these instructions and took the test seriously. They completed the test in about thirty minutes in all three classes.

The post-test utilized the same fifty seven multiple choice question as the pre-test. It was administered 27 days after the pre-test, at the end of the molecular biology unit. The addition of four free response questions tested the students ability to analyze, synthesize, and evaluate four major conceptual ideas presented in the unit: the central dogma, the transcription-translation process, the lac operon theory of genetic control in prokaryotes, and the kinds and effects of mutations. The students were informed about this post-test in advance. They were told that the results would not be used to determine their grades but would be used to measure what they had learned about molecular biology. The students completed the post-test in about one hour.

The data derived from the pre-test and post-test is summarized in Appendix F and will be discussed in the pages that follow. I analyzed the data for each of the three classes separately. By doing this I hoped to see if there was variation between the classes in their increase on the mean score of the test. Secondly, I pooled the data from the three classes to give an overall picture of the increase in knowledge achieved by the 46 students in the study. Finally, I did an item analysis of the number of students who missed

each test item to give me an understanding of the specific topics the students had failed to master. By doing this I hoped to learn how to modify the unit to make future instruction more meaningful in helping students construct their knowledge of molecular biology.

The third period class, which also met second period twice a week, was the class that I expected to show the least growth. This class was my "guinea pig" group for, due to scheduling, this class would be introduced to the unit one day before the other two sections. I assumed that this gap of one day in instruction would introduce slight modifications in the unit which would make the presentations more effective for the other two classes, permitting the students to be more effective in constructing their knowledge.

The third period class was the largest class with eighteen students, sixteen ninth graders and two sophomores. The lowest pre-test score in this class was four correct out of fifty seven, the lowest score in any class tested. The highest pre-test score was seventeen correct. The highest post-test score of this class was forty-seven correct while the lowest score was twenty-two correct responses. None of these scores were attained by the same students. The mean score of the pre-test was 12.27 with a standard deviation of 3.44, a variance of 11.83, and a standard error of the mean of 0.81. On the post-test the mean score rose to 35.38, with a standard deviation of 6.76, a variance of 45.70, and a standard error of the mean of 1.59. In comparing the pre- and

post-test results of this one class I found the standard error of difference to be 1.79. I applied a "student t" test and found the "t" value to be 12.91 with 34 degrees of freedom. Using a distribution of probability table I concluded that the probability of these two sets of test scores being different because of chance is less than 0.1%. Therefore I rejected the null hypothesis that the students did not show growth on the mean score.

The fourth period class, with a second meeting fifth period twice a week, included fifteen students. One of these students was an eleventh grade foreign exchange student from Germany. I have not included his test scores in the class summary because of his difficulties with mastery of English technical vocabulary. The remaining fourteen students were eleven ninth graders and three tenth graders. The lowest on the pre-test was eight while the highest score was twenty-one. The lowest and highest post-test scores were sixteen and forty-two respectively. The pre-test mean score was 12.2 with a standard deviation of 3.76, a variance of 14.13, and a standard error of the mean of 1.00. The post-test mean for this class was 32, with a standard deviation of 8.57, a variance of 73.44, and a standard error of the mean of 2.29. Comparing pre- and post-test results gave a standard error of difference of 2.03. Applying the "t test" to the pre- and post-test data yielded a t value of 7.92 with 26 degrees of freedom. Consulting the distribution of probability tables for critical values of t allowed me to conclude that the

probability of these test results being different merely due to chance was less than 0.1% Therefore I rejected the null hypothesis. Because the results of the third and fourth period classes were so similar, I also concluded that there was no difference in student growth on the mean score that was attributable to the one day gap in instruction.

Fifteen students were enrolled in the seventh period class, fourteen freshmen and one sophomore. The post-test of one of the ninth grade students was lost, so the data includes only fourteen students. The lowest score on the pre-test in this class was seven correct and this student also scored the lowest of any student tested on the post-test with a score of eleven correct. The highest score in this section on the pre-test was twenty-eight and this student also scored the highest score of any tested student on the post-test with a score of fifty-one correct. This class had the highest mean score on the pre-test 14.4, with a standard deviation of 5.15, a variance of 26.52, and a standard error of the mean of 1.37. The post-test mean grew to 32.35, with a standard deviation of 10.89, a variance of 120.56, and a standard error of the mean of 2.91. Comparing pre- and post-test results gave a standard error of difference of 3.24. The t value was 5.54 with 26 degrees of freedom. The probability of these scores being different merely due to chance was again less than 0.1% allowing me to reject the null hypothesis.

I also pooled the data of all three classes, a total of forty-six scores out of the pool of forty-eight students. The

mean score on the pre-test was 12.91 which rose to 33.43 on the post-test. The standard deviation for the pretest was 4.15, while that of the post-test was 8.66. The standard error of the mean for the pre-test was 0.61 while that of the post-test was 1.27. In comparing the pre- and pos-test results I found the standard error of difference to be 1.12. The t value was 14.55 with ninety degrees of freedom. The probability of these two sets of scores being different merely due to chance was again less than 0.1% allowing me to reject the null hypothesis. It is clear that students had shown growth on the mean score of the test.

On the post-test free response section, all of the students were able to write a response to the question "discuss the 'central dogma' of biology." About half of the students drew a flow diagram to help explain the concept, while the others wrote a paragraph containing the same information. Forty-one of the forty-six students in the sample were able to predict the corresponding polypeptide chain for a sequence of nucleotide bases on the sense strand of a DNA molecule using a table of anticodons. Thirty-nine students were able to compare the different types of mutations and their possible biochemical effects. Half of the students were able to explain the operon theory of genetic control using the lac operon model as an example. Another four students used the tryp operon model as an example of genetic control.

The results of the free response section of the test

showed that students were able to apply what they had learned in the unit. Since one of my constructivist goals was to test student understanding through application, I was pleased that the students showed mastery of the concepts tested at such a deep level.

Item analysis of multiple choice questions answered incorrectly by students was revealing. Forty-three of the forty-six students (93%) incorrectly answered a question which asked for an analysis of statements about mutations (question 43). Thirty-five of forty-six students (76%) were unable to answer a question which asked them to apply their knowledge of the size of DNA molecules and its information storage capacity to a model (question 4). Eighty-seven percent of the students were unable to place DNA replication in its proper position in the cell cycle (question 22). Seventy-eight percent of the students did not comprehend the 3′,5′ relationship between DNA and its RNA transcript (question 36). Eighty-five percent of the students did not comprehend the structural difference between purines and pyrimidines (question 18). Ninety-three percent of the students did not remember the scientist who had deduced the structure of the nucleotide (question 3).

The preceding data allowed me to identify concepts in the unit which students had not mastered. The poor performance on question 3 was due to my oversight in not including the information in my guided lecture. The lack of understanding demonstrated by student performance on question 18 is less explicable because this concept was included at three

different places in the unit. This concept will require additional reinforcement in future instruction. The results of question 4 and question 22 indicate additional concepts that will require new instructional strategies as the unit is taught to other classes.

Interestingly enough there was not one question that every student answered successfully, although some questions were routinely answered correctly. Ninety-four percent of students were able to pick the correct complementary strand for a given DNA sequence (question 20). Ninety-one percent of tested students knew that DNA has a linear sequence because of the position occupied by sugar and phosphates in the chain (question 17). Ninety-one percent knew the definition of codon and anticodon (question 38). Eighty-seven percent knew that uracil is the base found in RNA (question 33).

I had counted student incorrect responses to questions as a guide to what concepts the students had failed to master. I then used this data on incorrect responses to analyze student performance on questions in different taxonomic categories. Overall the students were more effective in answering knowledge questions than in answering comprehension and application questions. The mean number of incorrect responses regardless of the taxonomic classification of the question was 19. The mean number of knowledge questions answered incorrectly was seventeen or thirty percent. The mean number of application questions answered incorrectly was twenty-four, or forty-two percent. The mean number of

comprehension questions answered incorrectly was twenty-three, or forty percent.

The students were less effective overall, as shown by their mean score, in answering the multiple choice questions on the post-test than I had expected based on my previous experience in teaching similar material to students of this age. However, the students were more effective, based on their percentage of correct responses, than I had expected in dealing with the free response questions. These questions asked the students to analyze, synthesize, and make predictions. My goal as a constructivist teacher was to deal with deep understanding more than mere acquisition of factual information. I was pleased that the students showed a mastery of this deeper understanding.

New Teaching Strategies

Overall student interest throughout the unit was high. The students were engaged and involved learners during a period of the year (winter and early spring) when instruction is particularly difficult. The students enjoyed the labs and most of the learning activities. The atmosphere of the classroom was positive and the students expressed a sense of accomplishment. They realized they had mastered a very difficult topic, and they felt good about their achievement.

Approaching the study of molecular biology from an historical perspective of the research that has lead to our current understanding of the topic was effective. The textbook used in the classes (Biology:Third Edition, Mader) utilized this approach. The idea of organizing the historical material around eight questions (DNA Science, Micklos and Freyer) allowed the students to appreciate science as a quest where, to paraphrase Thomas Huxley, one stands on the shoulders of giants who have come before. Sixteen pre- and post-test questions (questions 2, 3, 5, 6, 7, 8, 9, 10, 11, 13, 14, 24, 27, 28, 29, and 30) evaluated the knowledge students had constructed about the historical experimentation leading to the current knowledge of DNA. The mean number of incorrect responses of the students on these questions was 20.8 compared to a mean value of 19 incorrect responses for

all questions. Many students commented that they had never before comprehended the collaborative nature of science with such clarity.

The laboratory activities were also successful in allowing students to construct their own knowledge. Nine questions (questions 15, 26, 40, 41, 47, 48, 49, 50, and 55) tested knowledge that students constructed mainly through laboratory instruction. The mean for incorrect responses for these questions was 18.8, compared to a mean value of 19 for all questions.

The modeling exercises were the most successful instructional tools of all. Sixteen questions (questions 15, 16, 17, 19, 20, 21, 23, 35, 36, 37, 38, 40, 44, 45, 53, and 57) tested knowledge constructed primarily through manipulatives and hands-on activities other than laboratory exercises. The mean number of incorrect responses on these test items was 15.3 compared to a mean value of 19 incorrect responses for all questions.

Concept mapping proved to be a powerful tool for meaningful learning. Although I have no data to support this conclusion, the students provided anecdotal evidence to bolster my claim. At first the students were reluctant to try such a technique. However, once we hit upon the idea of writing the concepts on "post-it-notes" which could be moved easily to new positions on poster board indicating new relationships, the students were eager to use the tool. Several student concept maps are reproduced in the appendix.

Students began to routinely utilize concepts maps for study and review. This was the strongest indication that concept mapping worked in helping them construct new knowledge.

The Vee heuristic served as a powerful tool for understanding knowledge and the construction of knowledge for many of the students. Those students who were operating at the higher cognitive levels, about sixty percent as shown by their scores on the DIQ, immediately grasped the significance of the instrument. They used the Vee in completing their laboratory evaluations. The remaining students, however, found the Vee to be confusing. "Maddening", and "stupid", were comments directed at my instructions to use the Vee in laboratory reports in this molecular biology unit. These students were the more concrete thinkers in my classroom. I concluded that the use of the Vee heuristic is extremely valuable for some students and probably worth the struggle for all.

A full evaluation of each laboratory exercise and modeling activity can be found in the preceding section on implementation of the unit.

Student Interviews

I interviewed three students before and after the introduction of the unit on molecular biology. The students represented the top, middle, and bottom of the achievement range of the seventh period class as determined from previous performance in class and from standardized testing (DIQ and Iowa Silent Test of Reading).

The highest achieving student was a ninth grader with a tested ability of 145 (DIQ) and who read in the top one percent of all students tested (Iowa Silent Reading, independent school norms). Indeed, this student had the highest pre- and post-test score of all tested students.

The middle achieving student, also a ninth grader, had a tested ability of 130 (DIQ) and read in the ninety-first percentile (Iowa, independent school norms). This student had an average pre-test score, and an above average post-test score.

The lowest achieving student had a tested ability of 123 (DIQ) and read at the fifty-seventh percentile (Iowa,independent school norms). This boy had one of the lowest pre-test scores, and was second from bottom in post-test scores. He is near or at the bottom of the ability range enrolled in this college preparatory independent high school.

I asked the students four questions in the preliminary

interview to assess their knowledge of molecular biology. The first question was, "What is DNA and what is its function in living things?" All of the students knew that DNA was involved in determining hereditary traits, probably as a result of the previous instructional unit on Mendelian genetics. All three of the students also knew that DNA is found in the chromosomes in the nucleus. All three students knew that for chromosomes to replicate the DNA molecule must also replicate. The high achieving student also knew that DNA controls protein synthesis at the ribosome through the action of RNA. The middle achieving student knew that DNA had something to do with proteins and that this "is your heredity" but he was unclear as to the details. The low achieving student told me that DNA is "in your genes," and "what kind of DNA" is in each cell "makes the cell different" from the others. This I found to be a common misconception shared by many students.

The second question I asked was, "How are proteins manufactured in cells?" All three students knew that ribosomes were the site of protein synthesis, a concept the class had studied in the unit on cell structure. The high achieving student was able to explain the central dogma of molecular biology although he did not know it by name. He also knew that proteins were polypeptide chains and that the sequence of the nucleotides in the DNA molecule specified the primary structure of the protein. He hypothesized that the ribosome "hooks up" amino acids in the sequence "ordered by

the DNA in the nucleus" and that this action requires RNA to be present. The middle level student and the low achieving student also knew that proteins were composed of amino acid units, but neither were able to explain how DNA was connected to protein synthesis, or what happened at the ribosome. Neither mentioned RNA. As a further extension I asked the three boys if they had ever heard the terms codon, anticodon, translation, or transcription applied to biology. None of the three had heard of these terms in a biological context.

The third question I asked in the preliminary interview was, "Tell me what you know about genetic engineering or recombinant DNA." The high achieving student had read several articles in the newspaper and popular magazines about genetic engineering. He also was familiar with the "ice minus" case, and could outline some of the ethical issues associated with releasing a bioengineered organism. This student also knew that viruses were used to "insert DNA into living cells" and he knew that the heredity of the cell was altered. He had an overall sense that genetic engineering was very useful and that it had great potential for doing good or harm. He brought up the topic of transgenic animals. He also mentioned gene therapy for curing "diseases."

The middle achieving student said he knew "a lot about genetic engineering." He knew that viruses were used as vectors to transfer genes. He also was aware that bacteria could "pick up" pieces of DNA from their environment. He defined recombinant DNA as a piece of DNA with a "not normal"

gene inserted into it. He also felt that this technology was dangerous because you never knew what "you might create in a test tube."

The low achieving student was aware of what genetic engineering meant in the broad sense. He defined it as "changing someone's genes to make them taller or smarter." He felt that DNA technology was dangerous although he was unable to articulate exactly why he felt that way.

The last question I asked the three students was not a content question. I asked the students to "tell me how you learn best." The high achieving student pictured himself as very good in math, science, and foreign languages. He described himself as good at memory tasks like memorizing list of words and definitions. He also felt that he wrote well. He studied by taking a textbook and "highlighting" the important passages. He felt that he took good notes in class and especially liked teachers who put outlines of the discussion on the overhead projector. He also liked "experiments" but wasn't especially fond of group work where he believed other students let him do all the work. When asked if he was good at conceptual tasks he responded that he loved to solve problems. He also saw himself as a visual learner because he learned best from textbooks. When I responded that reading is an auditory activity he quickly agreed that he did learn best by listening in class.

The middle achieving student saw himself as average across the curriculum. He read and took notes on the reading

but disliked class discussion preferring classes that were teacher directed (lectures and taking notes). He also saw himself as a visual learner who could learn best from pictures, educational films, and flow diagrams. He studied "hard" but was somewhat discouraged that he could never seem to do better than average work. He liked doing experimental work and hands-on activities but often couldn't see how the laboratory work had much to do with the reading that was assigned.

The low achieving student, not surprisingly, found school to be "too hard." He studied but found little reward for his studies. He liked activities, especially "outdoor stuff," and his favorite part of the biology course had been the fall field study of a forest community. He also liked experiments, but didn't see them as a learning tool. He especially disliked "those labs where you always have to write about what your conclusion is" because he never understood the nature of the task. He liked computers and used the computer to help with his homework. His handwriting was especially illegible and he often wrote even small assignments using a word processor. He didn't especially like to read and he found the biology textbook to be much too hard to understand. He liked classes where there was discussion as he was much better in oral expression than in written expression.

I interviewed the three students again at the completion of the unit. In the content area questions all the students showed remarkable growth. All three could now describe the

molecular structure of DNA in detail. All the students could describe the replication process and how DNA was related to chromosomes. The students also could explain in detail the central dogma of molecular biology, the process of transcription and translation, and the concept of one-gene-one polypeptide. They also could describe how the transformation of E. coli took place and how recombinant DNA was utilized in the process. They no longer saw genetic engineering as threatening, but as rather unremarkable and unspectacular.

When asked how they learned best, all three students described how concept mapping had helped them master the material. They were split on the value of the Vee heuristic, with the high achieving student finding it the most valuable in helping him construct learning from the laboratory activities. All three thought the laboratory activities were "interesting" and helped them learn more effectively. All three mentioned the DNA spooling laboratory and the gel electrophoresis laboratory as being effective in presenting knowledge. None of the three found the transformation of E. coli helpful in understanding the mechanism of genetic control in prokaryotes although it did help them appreciate what is meant by recombinant DNA. They also found the three DNA modeling exercises very useful in understanding DNA structure, replication, and transcription-translation.

DISCUSSION AND CONCLUSIONS

Aspects of the Unit Which Were Effective

The goal of the unit was to help students construct their own knowledge about molecular biology utilizing the five major principles of the constructivist pedagogy: (1) posing problems of emerging relevance to learners, (2) structuring learning around primary concepts, (3) seeking and valuing student's points of view, (4) adapting curriculum to address student's suppositions, and (5) assessing student learning in the context of teaching. An additional goal was to present DNA science in an historical context so that students might gain an appreciation of scientific inquiry as the process of discovery and subsequent modification of existing hypotheses.

The student modeling exercises were the most effective instructional strategies in accomplishing the unit goals. Student were most proficient in answering post-test questions which evaluated knowledge presented largely through visual and kinesthetic activities. Evaluation of student journal entries for questions posed by these activities also demonstrated the effectiveness of this methodology in helping students construct their own knowledge. Unexpected teaching opportunities such as a discussion on the shortcomings of models of reality also arose from this type of instruction.

These opportunities helped in achieving the goal of addressing student suppositions and in achieving the goal of seeking and valuing student opinions. Informal evaluation through student-student and student-teacher exchanges lead me to conclude that the students liked learning in this way.

Laboratory activities were also effective in achieving the goals of the unit as indicated by post-test results, student interviews, and informal evaluation of student journal entries. The students found their experiences in extracting and manipulating DNA to be a worthwhile learning experience. Their naive understanding of the nature and scale of the DNA molecule was replaced with a newly constructed concept much closer to reality. The restriction digest and electrophoresis of lambda DNA was the student favorite. It allowed them to visualize how DNA science could address problems in forensic science, an important conceptual leap that made bioethics a problem of emerging relevance to the boys as learners.

The structuring of the unit around major conceptual questions answered through historical experimentation in DNA science was also effective in helping students learn. Not only did the students come to an appreciation of the nature of scientific inquiry, but they also came to understand the evidence upon which our current understanding of molecular biology rests. This was apparent from their test results, but was most apparent in the student interviews and in informal group assessment. To me, this was one of the most important primary concepts in the unit. Far too often students naively

view science as a belief system one embraces rather than as an intellectual construct based upon evidence gathered through experimentation. This portion of the unit was very effective in challenging that naive student assumption and in helping them to construct a new understanding to replace the old.

The methodology utilized was also effective in meeting the goals of the unit. Students embraced concept mapping as a tool for everyday learning, an outcome I had ardently desired. Students worked effectively in cooperative learning groups to negotiate meanings and construct knowledge. The Vee heuristic became a powerful tool for some in achieving knowledge about how knowledge is constructed. It helped all students integrate the thinking side with the doing side. The class project, the building of a three dimensional model of the DNA code for proinsulin, was effective in helping students construct knowledge about the scale of DNA and protein as well as reinforcing the central dogma of molecular biology. It furthered my hidden curriculum agenda of promoting student cooperation through learning activities structured around individual accountability to the group.

The monitoring of student understanding through journal entries was also an effective teaching and learning tool. Students were accountable for what they had accomplished in laboratory and modeling activities. They were empowered to design their own model of assessment through the format in which they chose to present their understandings to me. By reading their journal entries overnight I was able to

immediately identify misconceptions and to address them in the next class period.

Entry and exit polls provided another tool of authentic assessment. Unlike quizzes, which tie understanding to performance, entry and exit polls provide an immediate measure of student understanding with no penalty for "not understanding." At first the students were puzzled by my use of these learning tools, but once they understood that I really wasn't grading these polls they were very open and honest in expressing their lack of understandings as well as in sharing their successes.

Aspects of the Unit Needing Improvement

Two portions of the unit were inappropriate for the target students.

The first portion of the unit needing adjustment was the laboratory on the transformation of E. coli. Most students were unable to recognize the blue bacterial colonies on the plate as evidence for the lac operon model of genetic control in prokaryotes. The students were able to recognize the transformation process as the bacteria became antibiotic resistant. Further, the students were able to mimic mastery of the lac operon in class discussion and in their own writing. However most students were unable to construct an understanding of the connection between the blue bacterial colonies and the operon model. I reluctantly concluded that most introductory biology students were not developmentally ready for this concept. I have moved consideration of this topic to a second year biology class.

The modeling activity "How Genes Make Proteins" I also deemed inappropriate for this target group. Student feedback on this activity was very negative. Students felt that the activity infantilized them and was unworthy of their time. This activity would be appropriate for sixth or seventh grade middle school students.

Overall Evaluation

I concluded that the molecular biology unit was successful in attaining the goals I set for the instruction. However, the time required to accomplish the unit was more than can be justified in an introductory biology class. Twenty eight days out of one hundred sixty instructional days is almost twenty percent of the days allotted for students to construct knowledge about the entire spectrum of biological knowledge. I do subscribe to the philosophy that we must teach less content but teach it more thoroughly and innovatively. Even with such a "less is better" orientation toward the biology curriculum, I will reduce the length of the unit on molecular biology to fourteen days in future years. The other fourteen days will be spent on the anatomy and physiology of the fetal pig which, although dense with content, is exceptionally high in student involvement and interest.

APPENDIX A

DAILY LOG OF ACTIVITIES

APPENDIX A

Daily Schedule of Instruction

Day 1: Administration of the pretest of 57 multiple choice questions (Appendix E)

Overview of the molecular biology unit.

Day 2: Lecture on Questions I, II, and III of the timeline of the discoveries in the development of DNA science (Appendix D)

Day 3: Laboratory Activity I: Extraction and Spooling of DNA (Appendix B)

Day 4: Discussion of previous day's laboratory findings

Continue discussion DNA timeline Questions IV and V

Day 5: Laboratory Activity II: Properties of DNA (Appendix B)

Day 6: Discussion of previous day's laboratory results

Discussion of timeline Question VI

Video: "Viruses, Bacteriophages, and the Hershey and Chase Experiment" (The Biochemical Society, London)

Day 7: Introduction of the structural components of DNA at the molecular level: deoxyribose sugar, phosphate, purine and pyrimidine bases, complementary base pairing, 3',5' orientation, 3.4 nm helix repeat, 10 base pairs for each turn of helix

Modeling Exercise I: DNA STRUCTURE (DNA Simulation

Biokit) (Appendix C)

DAY 8: Student mastery of concepts presented to this point was assessed with a quiz

DNA timeline Question VII was discussed:

Experimental evidence that supports the semi-conservative replication model was presented.

Modeling Exercise II: DNA REPLICATION (Appendix C)

Day 9: Discussion of the relationship of DNA to the structure of procaryotic and eukaryotic chromosomes

DNA timeline question VIII was discussed.

Day 10: In depth study of Beadle & Tatum's Neurospora experiment as an invitation to inquiry

Day 11: Introduction of Central Dogma

Discussion of transcription and translation: roles of RNA, mRNA, tRNA, ribosomes, amino acids; concepts of codons, anticodons, initiation, elongation, termination

Video: "The Mechanism of Protein Synthesis" (Biochemical Society, London)

Day 12: Modeling Exercise III: How Genes Make Proteins (Appendix C)

Discussion of mutation and the effects of substitution, deletion, frameshift

Introduction of Modeling Exercise IV: A Class Project: The building of a DNA sequence which codes for the molecule proinsulin (Appendix C)

Day 13: Discussion of regulation of protein synthesis. Jacob and Monod model. Concepts of operon, repressor, inducers, promoters, introns, and exons were introduced.

Day 14: Student co-operative learning groups prepared a group concept map of the central dogma utilizing a list of concepts generated by the group. The maps were exchanged between groups, evaluated, returned, and modified until the group was satisfied.

Day 15: Introduction of recombinant DNA and associated terminology: plasmid, cloning, restriction enzymes, recombinant DNA, hybridization, sticky ends, and palindromes

Day 16: Modeling Exercise V: A Paper Model of the Production of a Recombinant Plasmid (Appendix C)
Assembled the class project, a cardboard DNA sequence that codes for proinsulin

Day 17: Laboratory 3: Bacterial Transformation of E. coli with pUC18 (Appendix B)
Overview of the laboratory procedure and rationale.
Introduction of bacteriological techniques and required apparatus.
Practice using micropipette apparatus to measure and dispense microliter quantities.

Day 18: Laboratory 3 continued: preparation of competent cells. Addition of pUC18 plasmid.

Day 19: Laboratory 3 continued: counted colonies from

day 18. Preparation of x-gal/IPTG plates. Discussion of expected results and how to calculate transformation efficiency.

Day 20: Laboratory 3 continued: colony counting and calculation of transformation efficiency. Discussion of the Lac Operon model.

Day 21: Co-operative learning groups met to write a group laboratory report on the transformation experiment.

Day 22: Laboratory 4: Gel Electrophoresis: Restriction Mapping of DNA Fragments (Appendix B) Introduction of gel electrophoresis, preparation of agarose gels, how to load the wells, operation of the power supply, how to stain the gels

Day 23: Laboratory 4 continued: preparation of the restriction digest of lambda DNA. Prepared and loaded digest samples. Ran gel. (done two hours previous to scheduled class time) Stained gel and refrigerated overnight

Day 24: Measurement and analysis of DNA fragments. Preparation of group laboratory report. Class discussion of the recombinant DNA activities

Day 25: Continued class discussion of DNA activities. Introduction of DNA fingerprinting

Day 26: Discussion of the ethical implications of biotechnology

Day 27: Continued discussion of bioethics

Day 28: Post test and clinical interviews

APPENDIX B

LABORATORY ACTIVITIES

Laboratory 1

A Down and Dirty DNA Extraction

Introduction:

DNA is found in the cells of all living things. DNA is like a very long, uncooked piece of spaghetti in that it can bend just so far before it breaks.
When we look at it, DNA appears to be very flexible, but this is because it is so long in comparison with its thickness. DNA is stiff and brittle, and must be handled carefully to keep the strands from breaking.

Cells can be opened to remove the DNA they contain by using detergents. Since cellular membranes are made of a double layer of lipids with embedded protein molecules, detergents will disrupt (dissolve) the cell membranes in much the same way they dissolve fats and oils on our dishes.
As the cell membranes dissolve the cellular contents flow into the liquid medium in which the cells were growing.

In this experiment the source of cells will be the bacterium E. coli. Since bacterial cells do not have a nucleus, as the detergent disrupts their cell membrane, the DNA, dissolved cell membranes, and other cell components will become suspended in the growth medium (Luria broth). The DNA molecules are soluble in the water of the growth medium and cannot be seen in this "cellular soup." DNA is not soluble in ice cold ethanol. Adding ethanol to the "soup" will cause the DNA to precipitate out while all the other components remain in solution. The DNA can then be spooled out using a Pasteur pipette with a hook at the end.

Materials:

For the class:

incubator at 37° C

hot water bath at 70° C

For each lab group:

culture plate with E. coli
eyedropper
6 ml 95% ethanol
50% solution dishwashing detergent in water
ice bath
inoculating loop
Pasteur pipette with hook
2 culture tubes of Luria broth, 4 ml each
wax pencil

Procedure:

Day 1:

1. Obtain individual colonies of E coli from the culture plate provided to you. Use an inoculating loop to remove and suspend a 2 to 3 mm mass of E. coli in a 15 ml culture tube containing 4 ml of Luria broth. Label this "Tube 1."

2. Obtain another culture tube of Luria broth but do not add any bacteria to this tube. Label it "Tube 2."

3. Incubate both tubes overnight at 37° C in the classroom incubator.

Day 2:

1. Remove the tubes from the incubator and to Tube 1 and Tube 2 add 3ml of a 50% solution of dishwashing detergent in water. Shake each tube to mix.

2. Place Tubes A and B in the water bath set between 65-75° C. Keep the tubes in the water bath for 15 minutes. Note: The temperature is critical. Temperatures > 60°C is needed to destroy enzymes, but temperatures > 80°C will denature DNA.

3. Remove the tubes from the water bath. Immediately add 3ml of ice cold 95% ethanol by pouring it carefully down the side of each test tube so that the alcohol forms a layer that floats on top.

4. Let the ethanol sit undisturbed for 2-3 minutes. Bubbles will form and you will see the DNA precipitate out of the solution as white threads. Push the Pasteur pipette with the hook on the end through the alcohol layer into the interface of the two layers where you see the white threads. Stir very gently and turn the tip of the pipette. The DNA should stick to the tip of the glass pipette and you should be able to carefully wind the threads of DNA onto the glass. The DNA will look like whitish mucus. Do not mix the layers!

5. Compare the appearance of the two test tubes.

6. Optional activity. Observe the DNA under the low and high power of the microscope by preparing a wet mount. You may stain the DNA with one of the nuclear stains available in the laboratory. Draw a picture of the DNA in you laboratory journal.

Questions:

1. Explain the effects that detergent has on the cell membrane.

2. What properties of DNA are demonstrated by this laboratory?

3. What features of DNA structure account for its stiffness?

4. If DNA is stiff and rigid, why do the DNA strands on the glass rod appear so flexible?

Instructors Guide to the Laboratory:

Target Group: Introductory Biology Students, Grade 9 and 10

Time Frame: 20 minutes first day, 45 minutes second day

Teacher notes:

1. Students should be supplied with LB agar plates containing actively growing cultures of E. coli from which to make their overnight cultures. As an alternative students could be given two individual aliquots from a Luria broth culture previously established by the teacher which is in the log phase of growth. This would allow students to begin the procedure for the second day immediately.

2. Almost any dishwashing liquid works for this procedure. The procedure has been done with Palmolive, Dawn and Joy. A solution of sodium dodecyl sulfate, 1 g/100 ml of H_2O will also work.

3. Freshly frozen liver will sometimes produce a good yield of DNA and the experiment has been done with plant material such as onion and peas. The cellular debris must be filtered out of the plant material before the layering procedure is carried out.

References:

1. "A Sourcebook of Biotechnology Activities", 1990, The National Association of Biology Teachers.

Laboratory 2

Investigating the Properties of DNA

Introduction:

In this laboratory we will continue our investigation of the properties of DNA. After spooling DNA onto a glass rod as in the previous laboratory exercise, you will test the ability of DNA to be put back into solution. You will further test the denaturing of DNA and the effects of the enzyme deoxyribonuclease I or DNAseI on the DNA molecule. This enzyme breaks the bonds between nucleotides in the molecule.

Materials:

blended calf thymus
95% ethanol
ice bath
DNAse I
2 test tubes
glass stirring rods
hot water bath
Pasteur pipettes
transfer pipettes
NaCl solution (1%)
SDS solution (1%)

Procedure:

Part I: DNA spooling

1. Place 3 ml of blended calf thymus into a test tube.

2. Add 1 ml of 1% SDS solution and allow to stand for five minutes.

3. Carefully pour 3 ml of ice cold 95% ethanol down the side of the tube so the alcohol forms a layer on the top of the solution.

3. Allow the solution to stand for 2-3 minutes. Carefully dip the glass rod into the test tube, through the layer of alcohol and into the thymus solution. Pull the rod up into the alcohol layer. Fine white threads of DNA should appear on the tip of the glass rod.

4. Spool the DNA onto the rod. In your journal record the appearance of the DNA threads.

Part II: Reverse the DNA Precipitation

1. Place the rod with the DNA fibers from Part I into a test tube containing 3ml of a 1% NaCl solution.

2. Agitate the rod in the salt solution until the DNA fibers disappear. Allow the test tube to stand for the time directed by your teacher (30 min to 24 hours).

3. Attempt to respool the DNA onto the glass rod by carefully adding 3ml of ice cold 95% ethanol as a layer on top of the salt solution.

4. Record your observations.

Part III: Denaturing DNA

1. Place a fresh 3ml sample of DNA solution in a test tube.

2. Using a test tube holder place the test tube in a boiling water bath for 2 minutes.

3. Remove the tube with the DNA solution from the boiling water and place it in ice for five minutes.

4. Add 3ml of ice cold 95% ethanol to the solution so that it forms a layer on the top of the solution.

5. Try to spool the DNA onto the glass stirring rod. Record your observations of the spooling attempt.

6. Repeat steps 1 and 2 of the procedure with a fresh DNA sample.

7. Allow the solution to cool slowly at room temperature for five minutes.

8. Add 3ml of ice cold 95% ethanol to the solution as a layer and attempt to spool the DNA onto the stirring rod. Record your observations of the spooling attempt.

Part IV: Effects of DNAse on DNA

1. Place a fresh 3ml sample of DNA solution in a test tube.

2. Using a micropipette add 50ul of DNAse I to the sample in the tube and mix.

3. Allow the tube to stand for 10 minutes.

4. After ten minutes add 3ml of ice cold 95% ethanol to the tube.

5. Attempt to spool the DNA onto the glass rod. Record your observations of the spooling attempt.

Interpretation of Results:

1. In your laboratory journal prepare a data table summarizing the results of your experimentation.

2. Describe as many factors that influenced your results as possible.

3. What basic properties of DNA did you obtain evidence for in the laboratory.

4. Describe the action of DNAse on the DNA in solution and tell what evidence supports your hypothesis.

5. Optional activity. Prepare a Vee-heuristic of the experiment.

Instructor's Guide to Materials in the Laboratory:

1. Calf Thymus DNA solution: 15 grams of fresh calf thymus blended at high speed with 100 ml of nuclear buffer solution.
   Calf thymus is available from most large meat suppliers.

2. Nuclear Buffer solution: (Modern Biology Kit #1) contains 1M magnesium chloride, 1% NaCl, 1M Nonidet P-40, and Tris pH 7.5 (0.19 g of Tris HCl, 0.09g of Tris Base, 0.07 g of EDTA, dissolved in 200 ml of distilled water).
   Dilute 5ml of concentrated nuclear buffer in 500 ml of distilled water and refrigerate.

3. SDS solution: 1 g dissolved in 100ml distilled water.

Source of Materials:

Modern Biology
PO Box 97
Dayton, IN 47914-0097

Laboratory 3

Bacterial Transformation of *E. coli* with pUC18

Introduction:

Transformation means change. In molecular biology transformation refers to a genetic change in which bacteria take up free DNA from the environment and incorporate it into their own genetic material. The natural rate of transformation is very low. Molecular biologists have found procedures that augment the uptake of DNA so that this natural method of genetically engineering bacteria will occur more readily.

The bacteria *Escherichia coli* (*E. coli*) is an ideal organism for molecular biologists to transform. It is easily grown in culture. This bacteria is genetically rather simple and well understood. Its genetic material consists of one large circle of DNA between 3 and 5 million base pairs in length. This is only about 1/600th of the DNA in a human cell. The bacteria also contains some small loops of DNA called plasmids usually ranging between 5,000 and 10,000 base pairs, but sometimes larger. These plasmids exist separately from the bacterial chromosme, and are replicated within the bacterial cell during division. Plasmids contain genes that enable bacteria to live and grow in special environments. Some plasmids, for example, carry one or more genes that make the bacteria resistant to antibiotics. Molecular biologists use plasmids to introduce new pieces of genetic information into bacteria.

E. coli does not take up plasmids readily. Biologists have found that certain conditions help bacteria take up plasmids, a condition called "competence." These conditions are:

1. The bacteria cells are rapidly growing. This is called the "mid-log" growth phase.
2. The cells are treated with cold calcium chloride.
3. After the foreign plasmid DNA is added to the cells in calcium chloride the cells are "heat-shocked" by briefly heating them to 40-42°C.

Exactly how the treatment outlined above increases "competence" is not known. It is thought that the cold temperature stabilizes the membrane and that Ca++ ions shield the negative phosphate ions in the membrane. This allows the

DNA to pass through the membrane because the negatively charged phosphate groups of the DNA are not repelled.

The pUC series of plasmids are artificial plasmids that have been constructed from parts of naturally occurring plasmids and from a portion of the E. coli chromosome. The plasmid contains the gene for ampicillin resistance. Ampicillin is an antibiotic that prevents E. coli from constructing cell walls. The portion of the plasmid derived from the E. coli chromosome contains the gene for the enzyme beta-galactosidase. This enzyme is required for E. coli to utilize lactose as an energy supply. Some strains of E. coli are called lac-minus because they do not produce this enzyme and so cannot degrade lactose. If a lac-minus strain is transformed by a pUC plasmid it regains its ability to synthesize beta-galactosidase and become lac-positive.

Laboratory Objective:

To introduce the plasmid pUC18 into E. coli cells creating a population of bacteria that are resistant to ampicillin and to illustrate the action of the "lac operon."

Materials:

plasmid pUC18 (30ng in suspension)	ampicillin (25mg/ml)
LB broth (luria broth)	LB agar (luria agar)
2% calcium chloride	E. coli (JM101 strain)
X-gal/IPTG/Amp LB plates	inoculating loops
micropipets (10ul, 100ul)	microcentrifuge tubes
test tube rack	37°C incubator
40°C water bath	ice bath
loop spreader	5 ml pipette

Procedure:

Part I: The Preparation of Competent Cells

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

2. Add 100 ul of E. coli in the calcium chloride solution and mix.

3. Allow the cells to stand in the ice for 10 minutes. They should become competent at this point. (Cells can be stored under refrigeration for 24 hrs.)

Part II: DNA Uptake by Competent Cells

1. Obtain two microcentrifuge tubes. Label one tube "+ DNA" and the other "- DNA."

2. Place the microcentrifuge tubes on ice for 5 minutes.

3. Transfer 10 ul of plasmid pUC18 to the tube labeled "+ DNA."

4. Transfer 50 ul of the competent E. coli to both "+" and "-" microcentrifuge tubes and swirl.

5. Place both tubes on ice for 15 minutes.

6. Transfer both tubes to the 40°C water bath for 5 minutes.

7. Add 700 ul of LB broth to both tubes. Incubate the tubes in the 40°C water bath for 30 minutes. This allows the bacteria to recover and begin to express the plasmid genes.

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

9. Transfer 200 ul of the bacterial suspension from each microcentrifuge tube onto the appropriate plate.

10. Use the glass loop spreader to spread the bacteria evenly over the agar in each plate. Be sure to flame the loop before transferring it to the second dish to prevent carry over of bacterial cells.

11. Let the plates stand for 30 minutes at room temperature. Then invert the plates and place them in the incubator.

12. After 24 hours count the colonies on each plate and record the number in your journal.

Part III: The Expression of the "lac operon"

Background to the Procedure: The pUC18 plasmid also transforms E. coli to utilize a different source of energy for growth. When grown on LB+ plates with X-gal/IPTG (a histochemical substrate which forms a blue-colored product and is also an inducer of the activity), the bacteria will switch from using galactose as an energy source and utilize lactose instead. As the bacteria grow and metabolize the media will turn blue as an indicator of transformation.

1. Using the same competent cells from part II, step 4 of the above procedure (the competent "+" cells), place 200 ul of the transformed cells on an LB X-gal/IPTG/Amp plate.

2. Spread the cells over the surface of the agar using the glass spreading loop.

3. Allow to stand for 30 minutes and then invert the plate an incubate it for 24 hours.

4. Count the number of colonies on the plate and record the number and appearance of the colonies in your journal.

Interpretation of Results:

1. Draw a picture of the three plates used in parts II and III.

2. Count the number of colonies on each plate and prepare a data table of the results.

3. Calculate the following:

   a. transformants/ml (from LB+ Amp plates)

   b. transformants/ug of DNA=

$$\frac{\text{(transformants/ml)(ml of transformation mix total)}}{\text{ug of DNA used for the transformation}}$$

   This last figure indicates the efficiency of transformation.

4. In your journal prepare an explanation of this laboratory. In your discussion explain how the experimental evidence explains the concept of transformation. Use the Vee- heuristic.

5. Using the data from the experiment explain the concept of the "lac operon."

Instructor's Guide to Materials in the Laboratory:

LB= luria Broth (for one liter):

1. Mix together in an appropriately sized flask
   10 g tryptone
   10 g sodium chloride
   5 g yeast extract
   0.1g NaOH

1 liter distilled water
2. Autoclave for 15 minutes at 121°C and 15 psi.

LB plates:

1. To 500 ml of Luria broth add 7.5g of bacto-agar.
2. Heat to boiling and pour into sterile Petri dishes.

Ampicillin:

1. Add 0.5 g of ampicillin to 25 ml of distilled water
2. Pass the solution through a sterile 0.45 micron Millex filter.
3. Collect the filtrate in 1 ml aliquots which can be frozen for later use.

2% X-gal solution:

1. 0.16 g of X-gal (5-bromo, 4-chloro, 3-indolyl, b-d galactopyranoside) in 8 ml DMF solution (N,N dimethylformamide)

100mM IPTG solution:

1. Dissolve 0.07 g of IPTG in 3 ml sterile water.

LB X-gal/IPTG/Amp plates:

1. Autoclave 500 ml of LB+ agar.
2. As the solution cools add 2.5 ml of 2% X-gal, 0.5 ml of fresh 100mM IPTG (isoprpoyl b-d-thiogalacto-pyranoside), and 1 ml of ampicillin solution.
3. Pour a thin layer into sterile Petri dishes.

Source of Materials:

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

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

References:

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

Laboratory 4

Gel Electrophoresis: Restriction Mapping of DNA Fragments

Viruses are generally not considered to be living organisms because they lack basic cellular structure. Viruses also do not independently carry out the normal metabolic and reproductive functions of a cell. Viruses do exist as parasites of cells. They can "take over" the genetic machinery of a cell, resulting in the production of additional viruses.

Viruses have a relatively simple genetic makeup. Their genome contains only between 3 and 240 genes compared with the estimated 100,000 genes in a human cell. Molecular biologists have studied the DNA structure of viruses extensively. One of the most studied viruses is called bacteriophage lambda. A bacteriophage is a virus that infects a bacterial cell.

Bacteriophage lambda's DNA is approximately 48,514 base pairs in length (the genome of one of your cells is approximately 3 billion base pairs). In this laboratory you will cut the genome of lambda DNA with two restriction enzymes: EcoRI and BamHI. These restriction enzymes act as chemical "scissors" to cut the lambda DNA at specific places called restriction sites. These sites are recognized by enzymes because they are palindromes. A palindrome is a word or sentence that is exactly the same forward or backward. "Madam, I'm Adam," is an example of a complex palindrome. DNA also contains palindromes such as:

5′ G A A T T C 3′
3′ C T T A A G 5′

A restriction enzyme which cut between A and G would produce a staggered cut in this example of DNA. This staggered cut is called a "sticky end"

There are many different rerstriction enzymes. Each one of them has a different recognition site. EcoRI was the first restriction enzyme discovered. It is named for the bacterial species in which it was discovered, Escherichia coli RY13 and I because it was the first found in this species.

The restriction enzymes EcoRI and BamHI will each cut the lambda DNA into six fragments. You will run the fragments of

your digest in a gel along with fragments of DNA of a known size. You will then compare the results and identify the restriction fragments.

Materials:

electrophoresis chambers	agarose
power supply (24 to 50 volts)	distilled water
electrophoresis buffer	methylene blue stain
running dye	lambda DNA
EcoRI and BamHI	37°C water bath
ice bath	microcentrifuge tubes
micropipetes (5 ul, 10 ul, and 20 ul)	
masking tape	gel staining tray
optional: Polaroid camera and film	
light box	

Procedure:

1. Obtain 4 microcentrifuge tubes and label them 1 through 4.

2. Load the tubes according to the following matrix:

Tube #	EcoRI	BamHI	Digested DNA	Glycerol
1			10ul	5 ul
2	10 ul			5 ul
3	10 ul	10 ul		5 ul
4		10 ul		5 ul

3. Add 5 ul of lambda DNA to each tube except tube number 1.

4. Tap the tubes with you finger or vortex.

5. Incubate the tubes for 30 minutes in a 37°C water bath.

6. Prepare an agarose gel by adding 1.2 g of agarose to 100 ml of electrophoresis buffer. Bring to a slight boil (hot plate or microwave). Stir to insure that all the agarose has dissolved.

7. Allow the agarose to cool for two minutes. Then pour it into two trays with the well-forming combs about one inch from the end. Allow the gel to solidify.

8. When the gel has set, remove the comb and the tape from the edges of the gel tray.

9. Place one of the gels, on the tray, in the

electrophoresis chamber. Carefully pour in electrophoresis buffer until the buffer just covers the top of the gel.

10. Using a micropipete, load the wells in the gel with 15 ul samples as listed below. Use a new tip for each sample.

Well Number	Tube Sample	Material
1	1	predigested DNA
2	2	EcoRI
3	3	EcoRI and BamHI
4	4	BamHI
5	-	running dye
6	2	EcoRI
7	3	EcoRI and BamHI
8	4	BamHI

11. Cut off one corner of the gel as a reference point for the wells after the experiment is completed. In your journal prepare a diagram of the gel with the wells numbered and labeled with the materials they contain.

12. Connect the positive lead of the power source to the electrode terminal furthest from the wells. DNA molecules have a negative charge and will migrate toward the positive charge. Connect the negative lead to the second electrode terminal.

13. Turn on the power supply and watch for bubbles to form near the carbon rods. At 30 volts it will take about one and one half to two hours for the gel to separate the fragments. Progress can be monitored by watching the separation of colors in the running dye. When the first band of color in the running dye (the orange band) is 1 cm from the end of the gel you should turn off the power supply.

14. Remove the gel from the electrophoresis apparatus. Measure the distance each band of the running dye in lane 5 has moved from its original position in the well. In your journal prepare a second drawing of the gel after the electrophoresis was completed.

15. Stain the gel by placing it in the plastic tray provided. Cover the gel with 1% methylene blue stain. Avoid getting the stain on your skin. Allow the stain to remain on the gel for 15 minutes.

16. Wearing gloves remove the gel from the stain. Return the stain to the container provided. Fill the tray with distilled water and rinse the gel in the water for several minutes. Repeat the washing several times with fresh water.

17. Wrap the rinsed gel in plastic wrap and refigerate it overnight. This will allow the bands of DNA to develop.

18. Measure from the wells to the bands of dye found in each lane of the gel and record your measurements. Prepare a drawing of the stained gel in your journal or photograph the gel using the Polaroid camera, film, and the light box.

Interpretation of Results:

Background Information: The running dye contains three different dyes. Xylene cyanol which is blue-green moved a distance equivalent to 2800 base pairs. Bromthymol blue which is bluish-purple moves a distance equivalent to 250 base pairs. Orange-G, orange in color, moves a distance equivalent to 70 base pairs.

The predigested lambda DNA had been cut with the restriction enzyme HindIII yielding fragment sizes of 23,130, 9,416, 6,557, 4,361, 2,322, and 2,027 base pairs.

1. In your journal prepare a map of the banding pattern in each lane of the gel. Label lane 5 and lane 1 with the proper base pair lengths.

2. Prepare a semilog graph of the results of lane 5. Plot fragment size in base pairs versus distance the fragment moved in the gel.

3. Compare your results from the other lanes with this known graph line and estimate the size of the fragments produced by the EcoRI digest and the BamHI digest. Prepare a table of these estimates.

4. Compare your estimates of the fragments produced with the known standards for these two restriction enzymes. Were you able to resolve all the fragments? If not, what explanations can you offer to explain the differences?

5. Prepare a Vee-heuristic of this laboratory exercise.

APPENDIX C

MODELING ACTIVITIES AND CLASS PROJECT

MODELING EXERCISE I: DNA STRUCTURE

Introduction:

In most living organisms the carrier of the genetic information is deoxyribonucleic acid (DNA). The intricate structure of the DNA molecule carries the genetic code for inherited characteristics from one generation to the next.

The DNA molecule consists of thousands to millions of nucleotides bonded together in an interconnected chain. Nucleotides have three components: a sugar molecule, a phosphate group, and a nitrogenous base. In DNA the sugar is a five carbon molecule called deoxyribose. The deoxyribose sugars are linked together by phosphate groups at the number three and the number five carbon atoms of the sugars. The number three carbon end, or three-prime (3′) position, of one sugar is bonded by the phosphate group to the number five carbon, or 5" position of another sugar. This is repeated to form long polynucleotide chains. The DNA molecule has as its structural backbone two antiparallel sugar-phosphate chains. The two single DNA strands are interconnected by hydrogen bonds between nitrogenous bases. Of the four nitrogenous bases, adenine (A) and guanine (G) are classified as purines while cytosine (C) and thymine (T) are pyrimidines. Because of their molecule structure adenine bonds very specifically with thymine and cytosine only with guanine. It is the base pairing between strands that dictates the spiraling DNA structure commonly called the double helix.

Procedure:

Each group needs the materials listed in the chart:

Quantity per group	Kit component	Component designation
60	white beads	deoxyribose sugar
60	red beads	phosphate group
15	orange beads	adenine (A)
15	green beads	guanine (G)
15	blue beads	cytosine (C)
15	yellow beads	thymine (T)
30	clear connectors	hydrogen bond

Assemble 60 nucleotides by attaching a phosphate group (red bead) to the 5′ position of the deoxyribose sugar (white bead). The 5" position is the hole 180 degrees away from the peg of the bead. Attach any one of the four nitrogenous bases (A, T, C, or G) to the 1′ position of the same sugar. The 1′ position is the hole 90 degrees to the left of the peg. Separate the 60 nucleotides into 4 groups of 15 according to their nitrogenous bases.

Construct a single-stranded polynucleotide chain by attaching the phosphate group (red bead) of one nucleotide to the 3′ peg of the sugar (white bead) of another nucleotide. Use eight nucleotides with adenine, eight with guanine, seven with cytosine, and seven with thymine. Attach the nucleotides in nay order but maintain the phosphate group to 3′ attachment form.

To form the typical double-stranded DNA molecule, a complementary, antiparallel single strand of DNA nucleotides must be built and bonded with the first strand. The remaining 30 nucleotide units must be linked together in the following manner: the 3′ pegs of the new strand should be aligned in the opposite direction of the initial strand. The nucleotides should be attached so that the cytosine on the initial strand pairs with guanine on the new strand and vice versa. Thymine on the initial strand should be matched to new adenine nucleotides and vice versa.

Connect the complementary nitrogenous bases on the two single strands with the clear plastic connectors representing hydrogen bonds. This will form a double-stranded DNA molecule. The order of the nitrogenous bases in the DNA molecule codes for specific hereditary information. Rearrangement of the sequence of the base pairs will change the genetic code.

Gently twist the DNA model into the form of a spiraling rope ladder.

Disassemble your DNA molecule, rearrange the nucleotide order and change the DNA code. Build a new molecule remembering to align the two strands in opposite directions and to pair A with T and C with G.

Evaluation:

In your laboratory journal draw one of the DNA molecules you have built. Identify the major concepts introduced in this modeling exercise. Write a summary of this activity demonstrating that you have mastered the major concepts.

Reference:

"Student Guide: DNA Replication, DNA Simulation BioKit", Carolina Biological Supply Company, Burlington, N.C. 27215.

MODELING EXERCISE II: DNA REPLICATION

Introduction:

DNA carries inherited genetic information in the coded sequences of its nitrogenous bases. It is essential that DNA be exactly duplicated from one cell division to the next to maintain the hereditary code. The process of DNA duplication is called replication. For replication to occur, the DNA double helix must be unwound and separated into two single-stranded patterns of nucleotides called templates. DNA nucleotides which are complementary are brought to each template. Hydrogen bonds form to link the nucleotides of the new strand to the template. The new antiparallel strand grows in a 5′-to-3′ direction as opposed to the 3′-to-5′ template. The replication process produces
two double-stranded molecules from one initial molecule.

Procedure:

Each group needs the materials listed in the table below. Note the designation of each component.

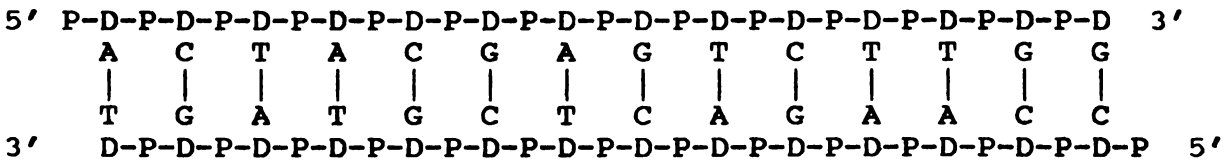
quantity per group	kit component	component designation
60	white beads	deoxyribose sugar
60	red beads	phosphate group
15	orange beads	adenine (A)
15	green beads	guanine (G)
15	blue beads	cytosine (C)
15	yellow beads	thymine (T)
30	clear connectors	hydrogen bonds

Assemble 60 nucleotides by attaching a phosphate group (red beads) to the 5′ position of the deoxyribose sugar (white beads) and by attaching any one of the four nitrogenous bases to the 1′ position of the same sugar.

By attaching the phosphate group of one nucleotide to the 3′ peg of the deoxyribose sugar of another nucleotide, construct the DNA molecule shown in Figure 1 below. Join the

two antiparallel strands with hydrogen bonds between the nitrogenous bases. Remember that adenine (A) always connects with thymine (T) and cytosine (C) with guanine (G).

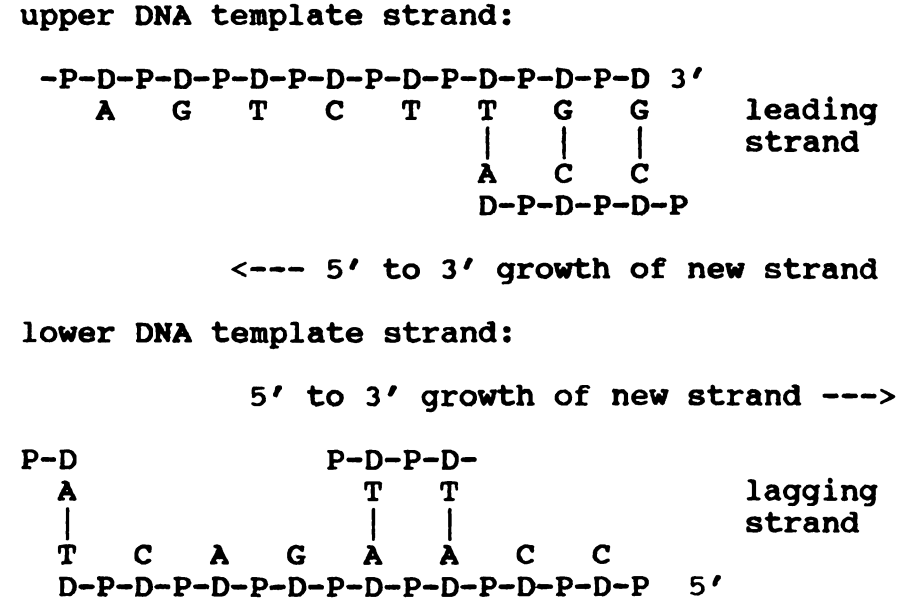
Figure 1 A simulated DNA segment



To show replication lay the double strands on your lab table as show in Figure 1. Place the 5′ end of the upper strand and the 3′ end of the lower strand on the left. The 3′ end of the upper strand and the 5′ end of the lower strand should be to the right.

Beginning on your right, unsnap the hydrogen bonds between the first eight pairs of nitrogenous bases and separate the two strands. Replication occurs in a 5′-to-3′ direction on a 3′-to-5′ template. Therefore new complementary nucleotides should be brought to the 3′ end of the upper template and positioned antiparallel to the nucleotides on the template strand. Place hydrogen bonds between the nitrogenous base pairs and attach the 5′ phosphate group of each new nucleotide to the 3′ peg of the last sugar on the growing strand. In the case of the first new nucleotide to be brought to the template, connect only the nitrogenous bases via a hydrogen bond. See Figure 2 below:

Figure 2 Separation of the two DNA strands and initiation of replication



Growth of the new strand proceeds one nucleotide at a time from the open 3′ end of the upper strand toward the separation point of the original single-stranded templates. Continue adding nucleotides until the template separation point is reached. This replicating strand proceeding toward the separation point of the two templates is called the leading strand. As the template separates the leading strand replicates continuously (see Figure 2, upper template strand).

On the lower template with the terminal 5′ phosphate group, replication moves away from the separation point of the two templates but still moves in a 5′-to-3′ direction. The lower or lagging strand must replicate in short, discontinuous segments to keep pace with the separating templates. To show this discontinuous replication bring the first complementary nucleotide to be attached to the lower strand to the fourth nucleotide from the 5′ end. The fourth nitrogen base on the lower strand is adenine and the new nucleotide to be hydrogen bonded must have thymine as its base unit. Replicate a short nucleotide segment by bonding the next three nucleotides to the right. Move up to the eight nucleotide on the lower strand (thymine) and bond an adenine nucleotide to the template thymine base. Add the next three bases to the right of this nucleotide. Continue pairing up the next three bases to the right until the first fragment of new nucleotide bases you added to the lower strand is reached (see Figure 2, lower DNA template strand).

Separate the remaining six nitrogen base pairs of the template strands by unsnapping the hydrogen bonds between them. Continuously replicate the upper leading strand by adding the 5′ phosphate group of a new complementary nucleotide to the 3′ peg of a nucleotide already on the new strand. Move up three nucleotides on the bottom strand and add a complementary nucleotide. Move to the right and hydrogen-bond two more nucleotides to the template to form another short fragment of the lagging strand. Finally move to the last nitrogen base (thymine) at the 3′ end of the lower strand and bond a complementary adenine-based nucleotide to the template. To complete the replication bond the last two complementary nucleotides to the right. The four short nucleotide fragments attached to the lower template should be bonded by snapping together neighboring 3′ sugars and 5′ phosphate groups present at fragment end points.

You should have two new antiparallel DNA double strands with 14 nucleotide pairs. Examine your two molecules. Are the two strands of each molecule antiparallel? Are all the nitrogen bases paired correctly? Are the two molecules identical? Correct any errors that are present.

Evaluation:

In your journal write a summary of this modeling activity. Show that you understand the concepts introduced.

MODELING EXERCISE III: HOW GENES MAKE PROTEINS

Purpose:

In this activity you will act out the steps in the transcription and translation process of protein synthesis.

Procedure:

I. The venue

The walls, floors, and ceiling of the classroom are analogous to the membrane of a cell. The windows and doors represent the membrane's selective permeable pores because they regulate the size of objects that enter and leave the cell.

The center of the room will represent the nucleus of the cell where transcription occurs. Enclose it with a circle of chairs and laboratory stools to represent the nuclear membrane. Spaces between chairs represent pores in the nuclear membrane that regulate the passage of mRNA.

The remainder of the classroom represents the cytoplasm of the cell. The area adjacent to the laboratory tables represents the ribosomes where translation occurs.

1. The teacher will distribute cards with DNA sequences and their complementary mRNA codes to two groups of students. The cards are arranged in groups of three letters because the nitrogen bases of the genetic code function as triplet-based units. The large letters on the card correspond to the first letter of the nucleotide base units A, T, C, and G. The base unit U is found replacing T in all RNA molecules.

II. Transcription

1. Assume that a strand of DNA has unzipped, exposing the DNA base units. In reality only one of the two strands of DNA is actively transcribed into mRNA. This strand is called the sense strand. You will be working with the sense strand in this activity.

2. Students with DNA cards should line up in the classroom area designated as the "nucleus" of the "cell." The student with the DNA card labeled TAC (the start sequence) should be on the left as the class sees him. The student with the ATC (stop sequence) should be on the right. All other DNA cards should be between these two and in any order.

3. The enzyme RNA polymerase catalyzes the pairing of DNA's exposed bases with complementary RNA bases. Students with RNA cards should match their three-letter sequence with the three-letter sequence of the DNA cards. The 3-base mRNA sequence is called a codon. RNA cytosine always pairs with DNA guanine. RNA uracil always pairs with DNA adenine. RNA adenine always pairs with DNA cytosine. RNA guanine always pairs with DNA cytosine.

4. After everyone has matched up their cards, use the following table to check the complementary base pairing. Except for the start and stop codons the triplets may be in any order:

DNA	TAC	GGC	TTA	CAG	CTC	GAT	AGG	CCG	ATC
m RNA	AUG	CCG	AAU	GUC	GAG	CUA	UCC	GGC	UAG
	start								stop

5. Students with DNA cards should sit down, leaving a chain of RNA sequences. You have simulated the process of transcription by making a short section of RNA that is complementary to DNA.

III. Translation

1. Students who previously had DNA cards should get tRNA cards and amino acid cards from the teacher. You should randomly scatter yourself in the "cytoplasm."

2. Students with tRNA cards should find their proper amino acids. Trade cards so that each student has one tRNA card and the proper amino acid.

3. The students holding the mRNA cards should leave the "nucleus" and move to the area designated as "ribosome."

4. Students with proper tRNA and amino acid cards should move to the corresponding mRNA card using the rules of complementary base pairing. Use the following table to check the mRNA/tRNA/amino acid pairings (except for the start and stop codons, the tRNAs and amino acids may be in nay order):

mRNA	AUG	CCG	AAU	GUC	GAG	CUA	UCC	GGC	UAG
tRNA and amino acids	UAC met	GGC pro	UUA asn	CAG val	CUC glu	GAU leu	AGG ser	CCG gly	AUC stop

5. As each tRNA anticodon finds its corresponding codon on the mRNA strand, the tRNAs detach from their amino acids. The

amino acids remain at the ribosome and form a peptide bond with the amino acids brought by the previous tRNA. Two or more amino acids linked in this way are called polypeptides. A protein is many polypeptide chains linked together.

Evaluation:

In your journal answer the following questions:

1. What is the difference between a DNA sequence of bases and an RNA sequence of codons?

2. How does DNA determine the arrangement of amino acids in a polypeptide?

3. Describe transcription.

4. Describe translation.

5. Use the following tRNA/amino acid relationships:

GGC	UUA	CAG	CUC	GAU	AGG	CCG
pro	asn	val	glu	leu	ser	gly

Complete the following table:

DNA: TAC AGG GGC CTC TTA CAG CTC GAT AGG CCG GAT ATC

mRNA:

tRNA:

amino acid: met stop

6. Using the same tRNA/amino acid relationships as above work backwards from protein sequence to DNA code:

amino acid: met leu val pro gly asn ser glu glu stop

tRNA:

mRNA:

DNA:

Reference:

A Sourcebook of Biotechnology Activities, National Association of Biology Teachers, 1990.

MODELING EXERCISE IV: A CLASS PROJECT

Introduction:

In this exercise the class will build a three dimensional cardboard model of the DNA molecule which codes for the production of a small protein molecule. The protein molecule we will use is proinsulin, a protein that undergoes modification after transcription to become the better known molecule insulin. The proinsulin molecule is a chain of 84 amino acids, which are listed and numbered in order below in Figure 1. The DNA sequence which codes for the production of this small protein is therefore 252 nitrogen base units in length, excluding the start and stop commands. Each group of students will build a portion of the sense strand of the DNA molecule which codes for proinsulin, and will then build the complementary half to the sense strand to arrive at the double helix.

Figure 1. Amino acid sequence of proinsulin

```
 1                                        10
phe-val-asn-gln-his-leu-cys-gly-ser-his-leu-val-glu-

                            20  21
ala-leu-tyr-leu-val-cys-gly-glu-arg-gly-phe-phe-tyr-

             30
thr-pro-lys-ala-arg-arg-glu-ala-gln-asn-pro-gln-ala-

 40                                          50
gly-ala-val-glu-leu-gly-gly-gly-leu-gly-gly-leu-gln-

                                60          63   1
ala-leu-ala-leu-glu-gly-pro-pro-gln-lys-arg-gly-ile-

                                10
val-glu-gln-cys-cys-thr-ser-ile-cys-ser-leu-tyr-gln-

                   20
leu-glu-asn-tyr-cys-asn
```

Procedure:

Each group will be assigned a portion of the proinsulin molecule as their responsibility. After you are assigned your amino acids, work backwards through the translation and

transcription process to arrive at the DNA code. Use the table of codons in your textbook to help with this process.

Assume that the DNA strand you have deduced is the sense strand and is the right half of the DNA double helix. Work out the complementary strand to the sense strand, remembering that it must run antiparallel to the sense strand.

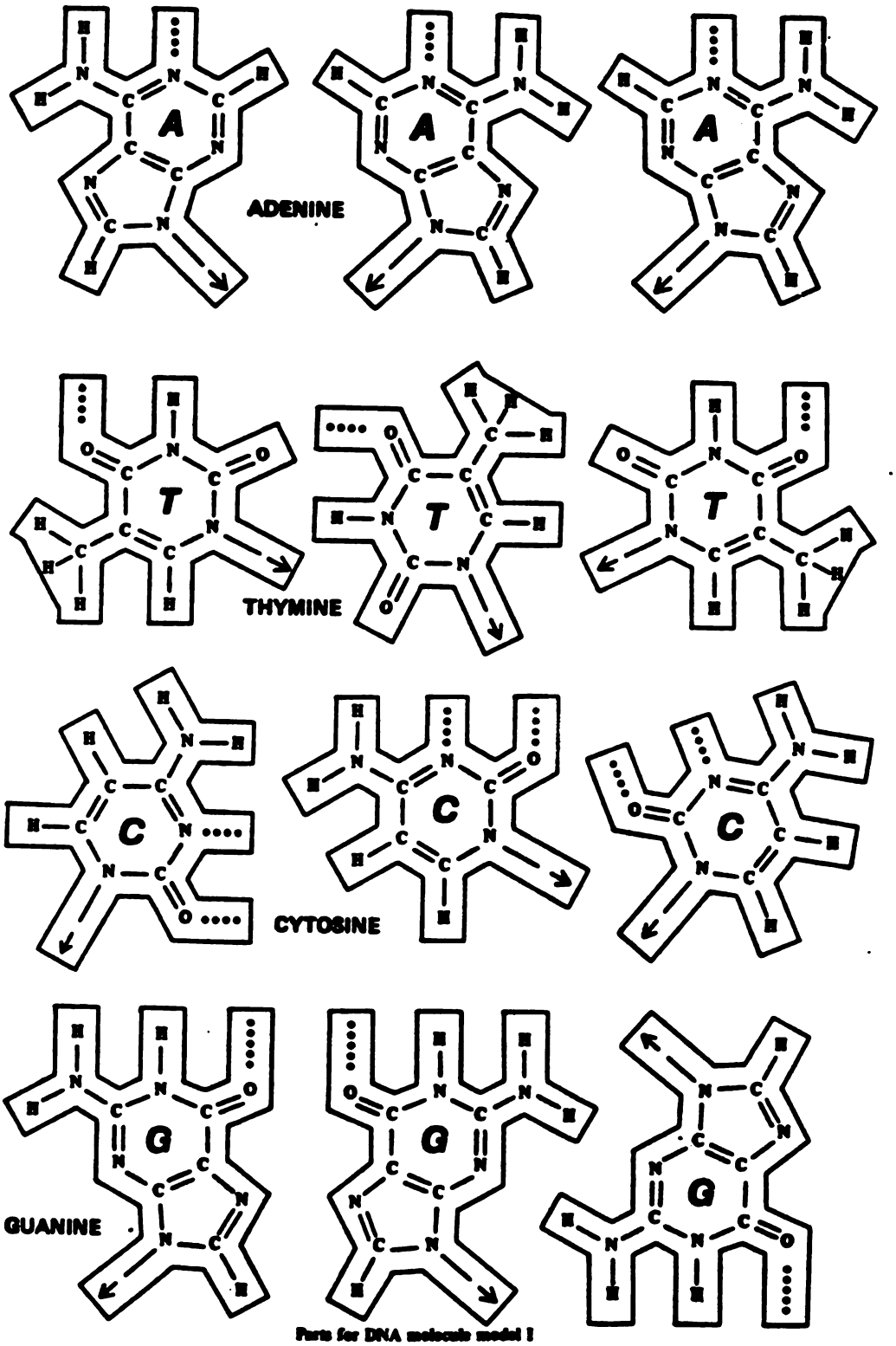
In the laboratory you will find sheets of paper on which are printed the structural formulas of the component parts of the DNA molecule. There are six different sheets in six different colors, representing adenine, thymine, cytosine, guanine, deoxyribose sugar, and phosphate groups.
The points of attachment of these component molecules are labeled. For the nitrogenous bases the arrow (------->) indicates the point of attachment to the sugar while the dotted line(s) (.....) indicate(s) the hydrogen bonds between the complementary base units of the two strands of the DNA double helix. The sugar molecules indicate where phosphate groups (PO_4) attach while the remaining arrow shows the attachment point of the sugar and nitrogenous base.

Paste these colored sheets onto light cardboard and then cut out the units. Prepare the proper nucleotides by attaching sugar, phosphate, and base units together to form the portion of the DNA molecule which is your responsibility. Glue the tabs together and then staple them for good measure. The molecule will twist when assembled and your joining must be secure. Remember the sense strand is the right strand. Be sure to label which end is which and be sure the two strands are antiparallel. Some of the sugars are right-handed sugars and some are left-handed sugars to help you in your modeling.

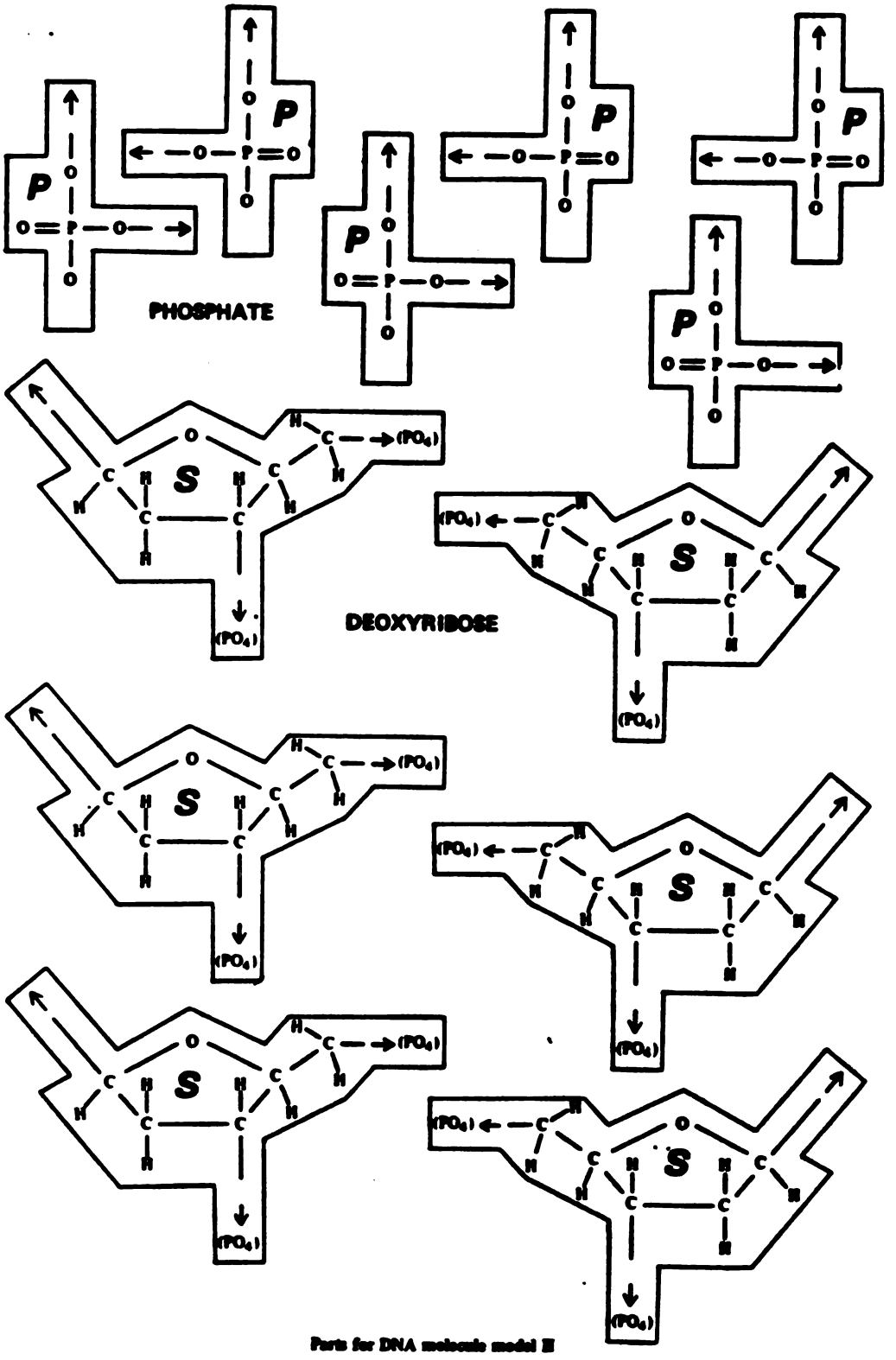
Bring your portion of the molecule to class on the assigned day. By putting the various portions together in the proper sequence we will have a very large illustration of the DNA molecule needed to code for a very small protein molecule. We will hang this model from the ceiling across the classroom.

<u>Reference</u>:

"Problem Solving in Biology", Eugene H. Kaplan, MacMillan Publishing Company,New York, 1983.



Parts for DNA molecule model I



Parts for DNA molecule model II

MODELING EXERCISE V:

A PAPER MODEL OF THE PRODUCTION OF A RECOMBINANT PLASMID

Background Information:

Current biotechnology utilizes three technologies in which living organisms carry out chemical processes or produce some useful substance. The three are bioprocesses, production of monoclonal antibodies, and recombinant DNA technology.

Bioprocesses are used in the manufacture of cheese and yogurt, the brewing and baking industries, and in the breakdown of sewage.

Monoclonal antibody technology allows the fusion of rapidly dividing cancer cells with antibody producing cells to form new cells called hybridomas. These cells can be cloned to produce large quantities of antibodies.

Recombinant DNA technology introduces a gene coding for a particular protein into a bacterial host. The host then expresses the gene as a protein product and as the bacterium multiplies the protein is produced in large quantities.

Purpose:

In this activity you will prepare a paper model of a recombinant DNA plasmid.

How recombination is accomplished:

Step 1: Identify and isolate the gene that codes for the production of the required protein. This is often accomplished by working from the amino acid sequence of the protein backwards through translation and transcription to the DNA sequence. The DNA sequence must then be removed from the host chromosome and isolated. This isolation is accomplished by the use of endonucleases (restriction enzymes) derived from bacterial cells. Endonucleases are normally used by bacteria to destroy foreign DNA that enters the cell. These enzymes can recognize and cut specific sequences of DNA at points on the DNA called palindromes. The cut is a staggered one producing a sticky end. These sticky ends can bind with complementary base pair sequences on other strands of DNA cut with the same endonuclease. The endonuclease can cut on either side of the desired DNA sequence.

Step 2: Insertion of isolated DNA into a plasmid. To insert the gene into the host bacterial cell a vector (agent

of insertion) called a plasmid is utilized. Plasmids are small, circular, extrachromosomal pieces of bacterial DNA. They are normally found in many bacteria and are part of the sexual reproductive machinery of bacterial cells. Plasmids have a region called the origin of replication from which they replicate. Plasmids are removed from bacterial cells and cut with the same endonuclease used to isolate the desired DNA sequence. Thus the sticky ends of the isolated DNA will match the sticky ends of the cut plasmid. Mixing cut plasmid and isolated gene together allows the isolated DNA to bond with the cut ends of the plasmid, thus closing the circular plasmid ring. This annealing process is aided by the action of DNA ligase, an enzyme which helps complete the bonding process.

Step 3: Insertion of the plasmid into the bacteria. The newly constructed plasmid is mixed with the host bacteria under conditions that will promote the uptake of the plasmid by the bacterial cells. This process is called bacterial transformation. To confirm that the host bacteria has taken in the plasmid the bacteria is tested for some basic characteristic the plasmid imparts to the bacteria such as antibiotic resistance by growing the bacteria in a medium which contains an antibiotic. Only bacteria which contain the plasmid will grow under these conditions.

Procedure:

1. Construct the Plasmid. The strips on the plasmid sheet are printed with the DNA 3′ to 5′ from top to bottom on the left-hand side of the strip and from 5′ to 3′ from top to bottom on the right-hand side of the strip. Cut of the strips of plasmid DNA and connect them in any order. You may omit a strip if you desire but be sure to include the origin of replication. Tape the plasmid into a circle.

2. Locate the Restriction Sites. Use the enzyme sheet to compare the sequences of base pairs on the enzyme cards with the base pairs you have on your plasmid. This will identify the restriction sites, the points at which the enzymes could cut the plasmid DNA. Mark a starting point of the plasmid and work around the circle marking the restriction sites as you go.

3. Prepare a Plasmid Map. Draw a map of the plasmid marking restriction sites, the origin of replication, and the sites that will confer antibiotic resistance in relative distance to one another.

4. Identify and Isolate the Gene to be Inserted. On the cell DNA sheet cut out the DNA strips 1 through 6 and assemble them in that order. This gene begins with the code for the amino acid methionine and ends with a stop codon. Determine which restriction sites are above and below the gene. Match

these restriction sites to the restriction sites you have identified on the plasmid. Use of the enzyme cards to cut the cell DNA above the gene and then cut the plasmid with the same enzyme. Find a second enzyme that will cut the cell DNA below the gene and then cut the plasmid with the same enzyme. The remaining portion of the plasmid must contain the origin of replication and at least one site for antibiotic resistance.

5. Assemble the Plasmid. Insert the cell DNA gene into the plasmid fragment using the DNA ligase enzyme card to catalyze the reaction. Bond the corresponding sticky ends together completing the plasmid.

6. Antibiotic Resistance for Detecting the Plasmid. To which antibiotic or antibiotics would this plasmid be resistant?

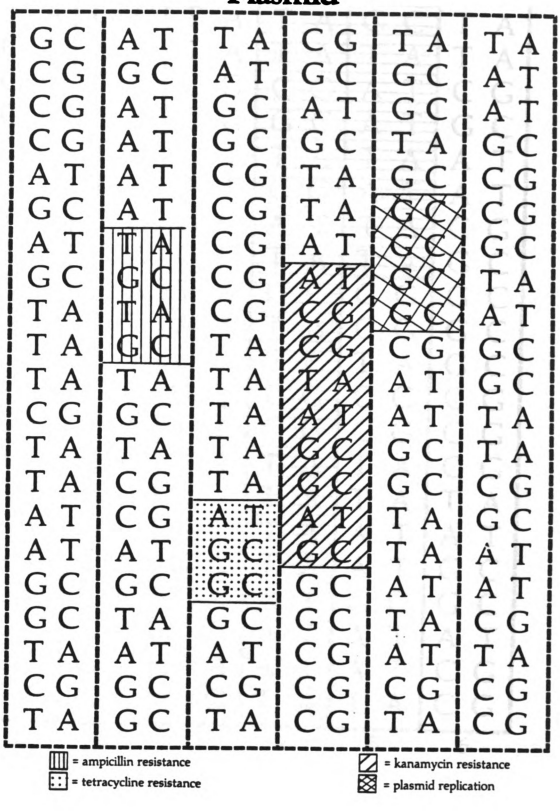
Evaluation:

Answer the following questions in your journal:

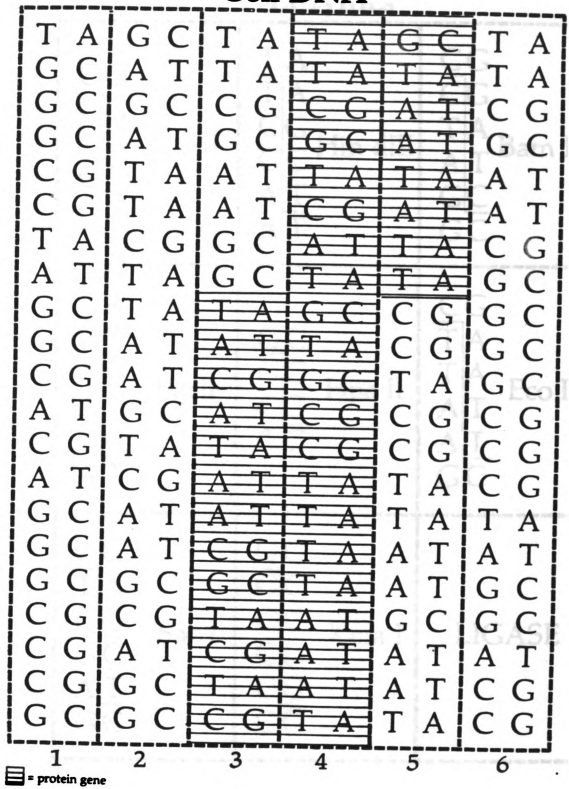
1. Which restriction enzymes did you use?
2. Which antibiotics would detect the plasmid?
3. What does recombinant DNA mean to you?
4. What are plasmids?
5. What are sticky ends?
6. What is a restriction enzyme?

Reference:

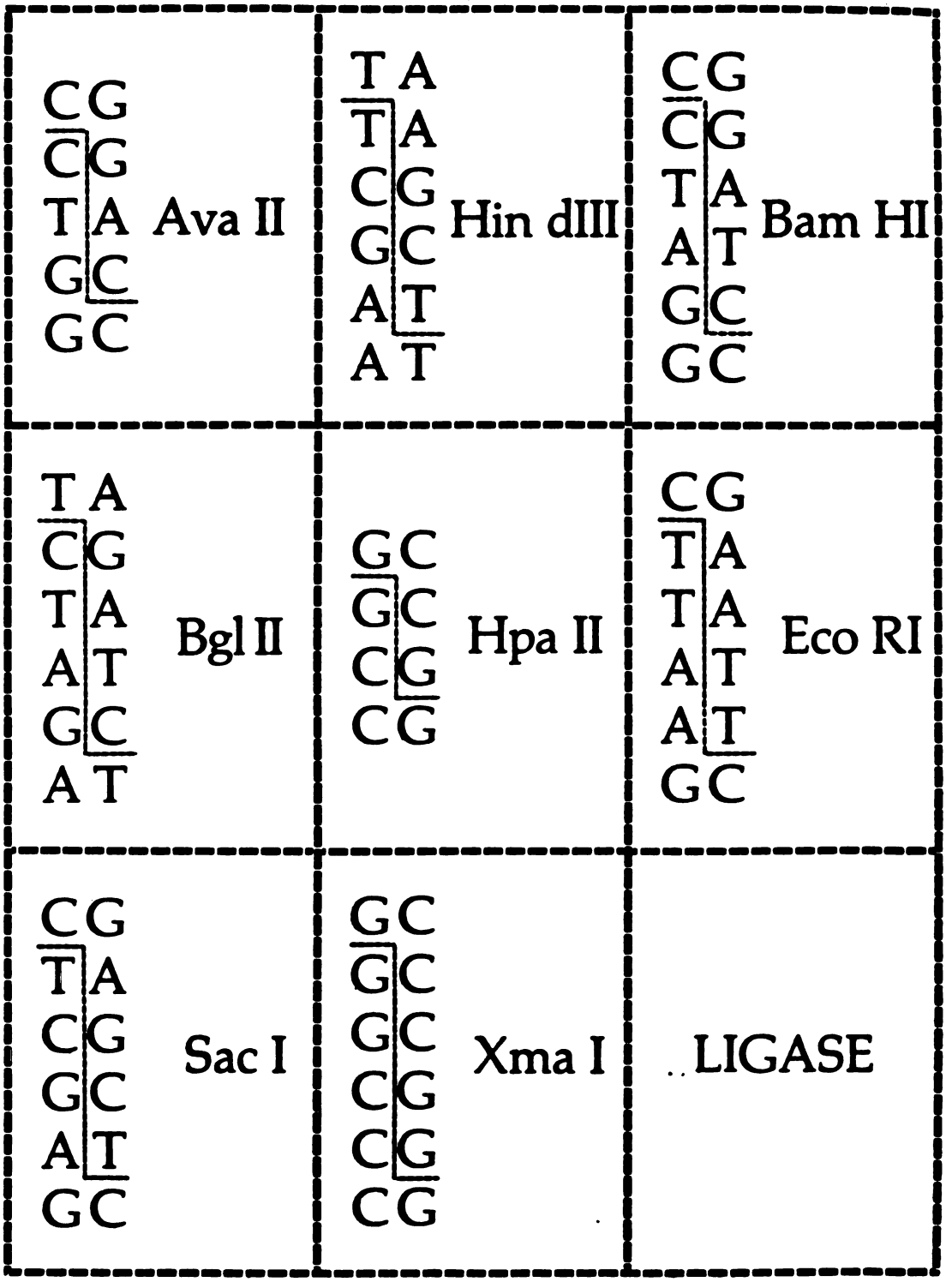
Adapted from "Recombinant Paper Plasmids", Christie L. Jenkins, The Science Teacher, April, 1987, p. 44-48.


Plasmid
= ampicillin resistance
= tetracycline resistance
= kanamycin resistance
= plasmid replication

Cell DNA



Enzymes


CG
CG
TA
GC
GC
Ava II
TA
TA
CG
GC
AT
AT
Hin dIII
CG
CG
TA
AT
GC
GC
Bam HI
TA
CG
TA
AT
GC
AT
Bgl II
GC
GC
CG
CG
Hpa II
CG
TA
TA
AT
GC
Eco RI
CG
TA
CG
GC
AT
GC
Sac I
GC
GC
GC
CG
CG
CG
Xma I
LIGASE

APPENDIX D

OVERLAYS

Figure 1

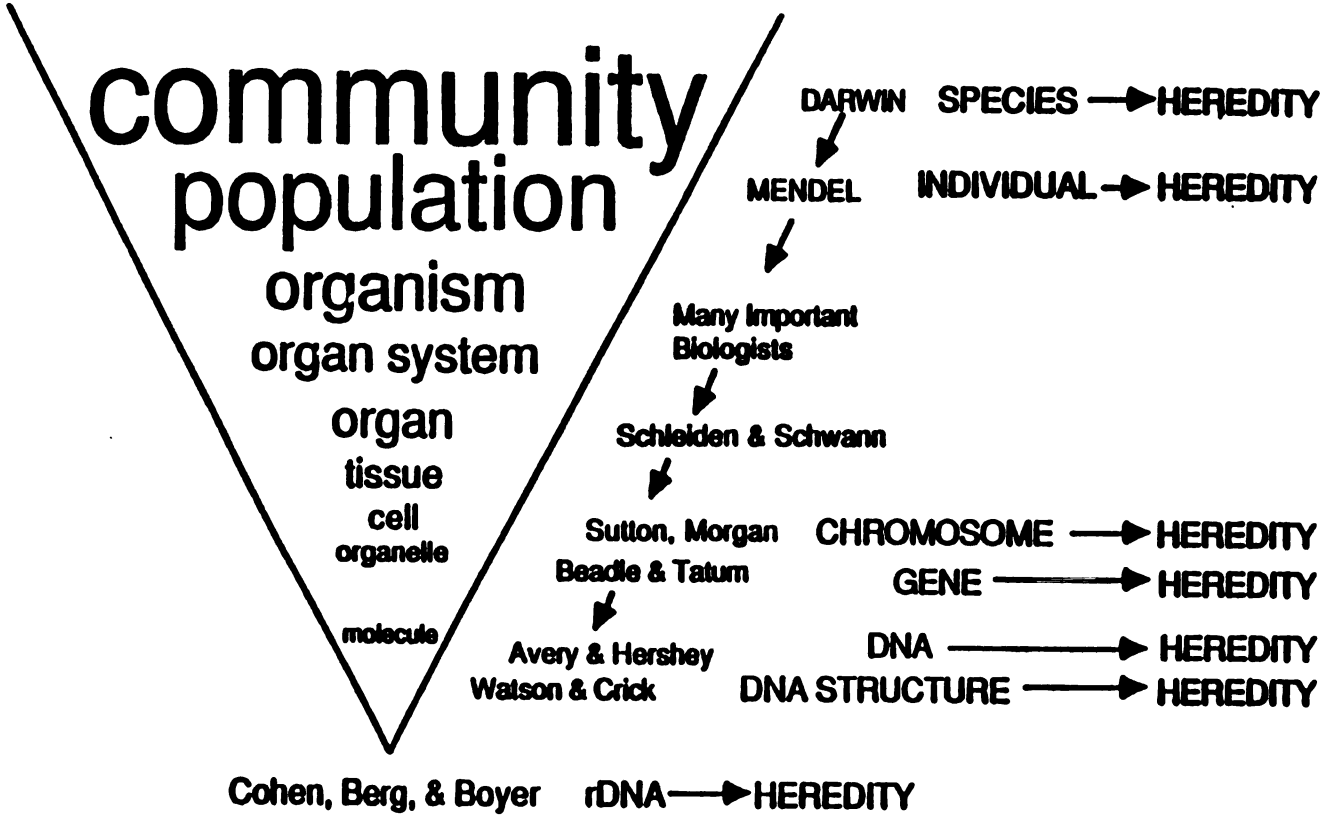
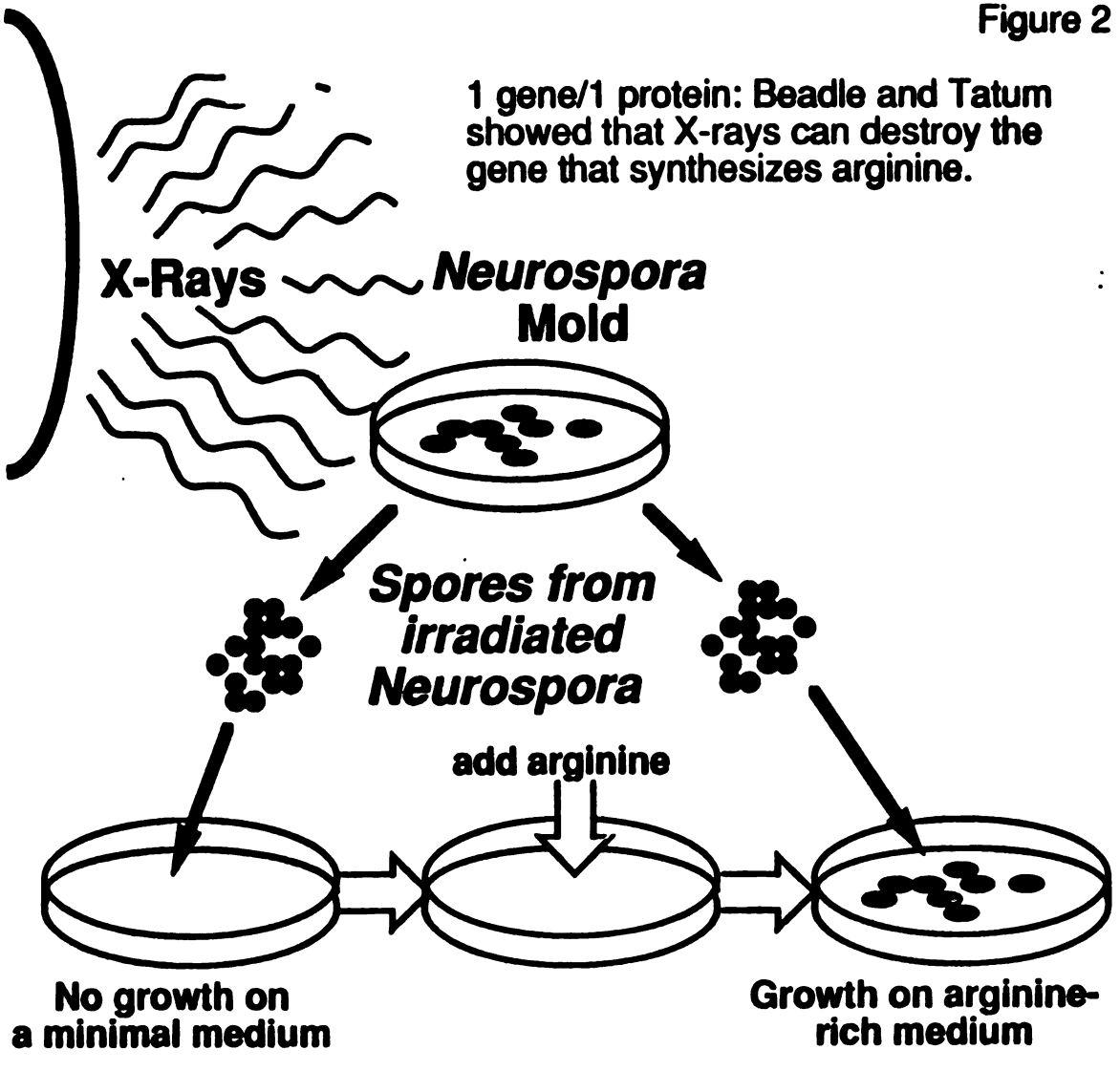

community
population
organism
organ system
organ
tissue
cell
organelle
molecule
DARWIN SPECIES → HEREDITY
MENDEL INDIVIDUAL → HEREDITY
Many Important Biologists
Schleiden & Schwann
Sutton, Morgan CHROMOSOME → HEREDITY
Beadle & Tatum GENE → HEREDITY
Avery & Hershey DNA → HEREDITY
Watson & Crick DNA STRUCTURE → HEREDITY
Cohen, Berg, & Boyer rDNA → HEREDITY

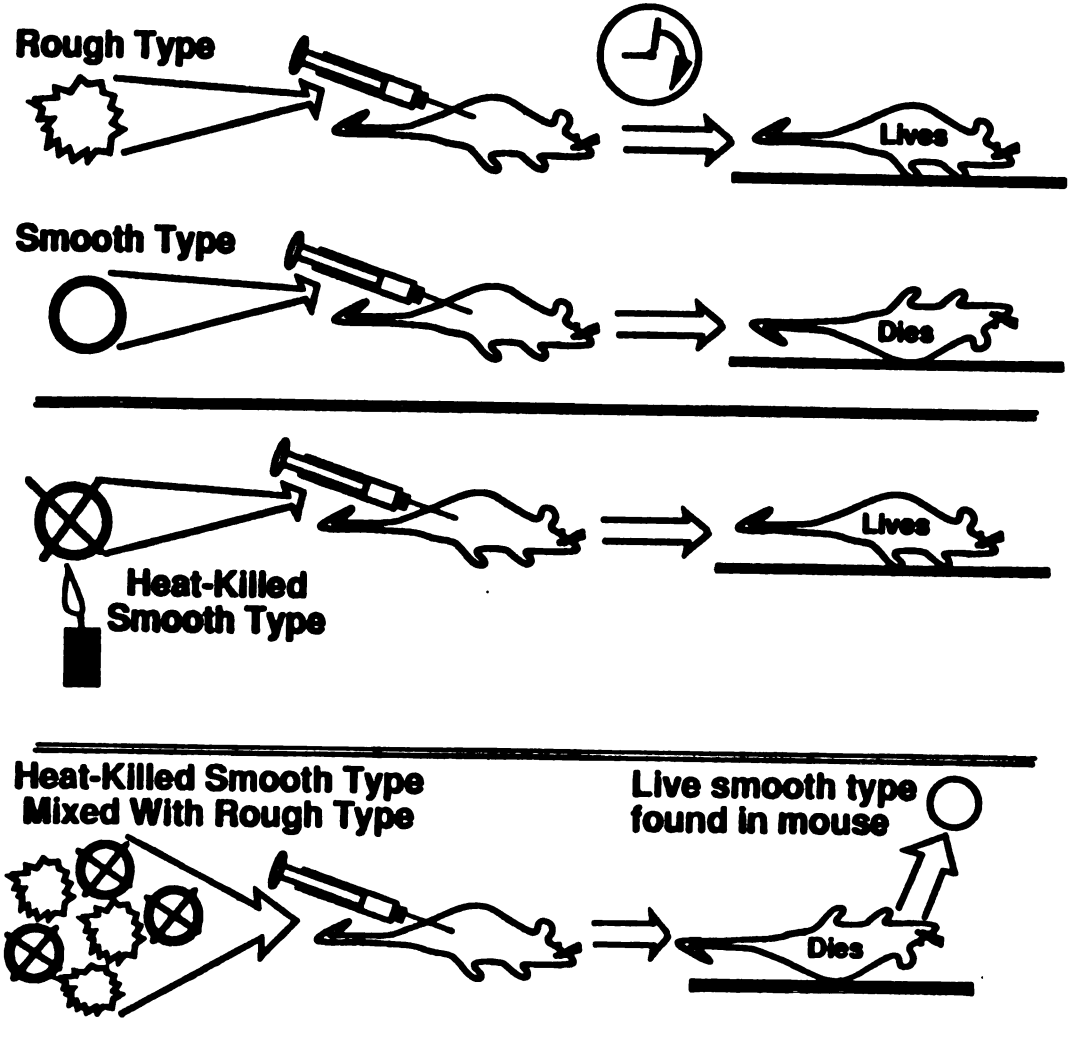
Figure 2


1 gene/1 protein: Beadle and Tatum showed that X-rays can destroy the gene that synthesizes arginine.
X-Rays
Neurospora Mold
Spores from irradiated Neurospora
add arginine
No growth on a minimal medium
Growth on arginine-rich medium

Griffith's Experiment:

A "transforming principle" exists in smooth pneumonia bacteria.

Figure 3



Hershey's Blender Experiment:

Figure 4

Phages descended from parents with radioactive DNA also have radioactive DNA.
Radioactive protein is not passed to offspring

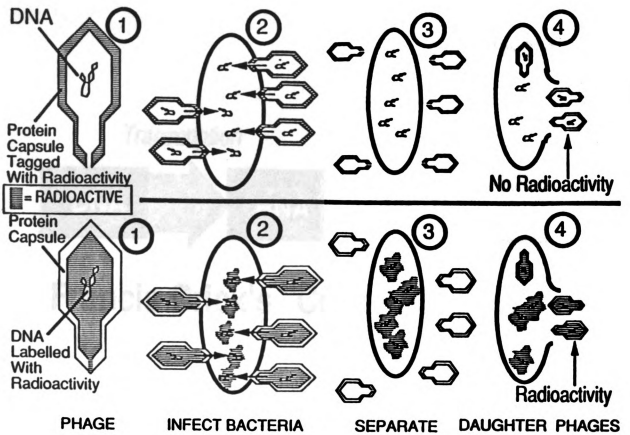
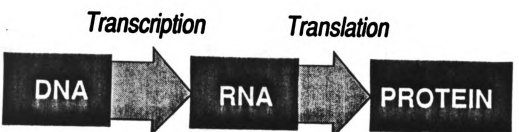


Figure 5



Francis Crick's "Central Dogma"

THE HISTORY OF DNA: A TIMELINE

Questions in the Development of DNA Science

I. How do we account for the diversity and similarity of species?

Scientist	Date	Experiment or Contribution
1.Linnaeus		
2.Wallace & Darwin		

II. How are traits passed from one generation to the next?

Scientist	Date	Experiment or Contribution
1.Mendel		
2.DeVries & Correns		

III. Where are these factors located?

Scientist	Date	Experiment or Contribution
1.W. Sutton		
2.N. Stevens & E. Wilson		
3.T.H. Morgan		
4.A. Sturtevant		

IV. What is the job of the gene?

Scientist	Date	Experiment or Contribution
1.A. Garrod		
2.H. Miller		

3.Beadle & Tatum

V. What molecule is the genetic material?

Scientist	Date	Experiment or Contribution
1.J. Miescher		
2.Fred Griffith		
3.E. Schrodinger		
4.Avery, MacLeod,& McCarty		
5.P. Levene		

VI. What is the structure of the DNA molecule?

Scientist	Date	Experiment or Contribution
1.M. Delbruck & S. Luria		
2.A. Hershey & Martha Chase		
3.Erwin Chargoff		
4. M. Wilkins & R. Franklin		
5. L. Pauling		
6. J. Watson & F. Crick		

VII. How does the structure of DNA allow replication?

Scientist	Date	Experiment or Contribution
1. M. Meselsen & F. Stahl		
2. A. Kornberg		

VIII. How does DNA allow protein synthesis?

Scientist	Date	Experiment or Contribution
1.		
2.		
3.		

APPENDIX E

PRE-TEST AND POST-TEST

MOLECULAR BIOLOGY

Pre-Test and Post-Test

1. Chemical analysis of eukaryotic chromosomes indicated that they consisted of:
   A. ribonucleic acid.
   B. protein.
   C. carbohydrate.
   D. deoxyribonucleic acid and protein.
   E. carbohydrate and protein.

2. DNA was first isolated in 1896 by:
   A. Robert Feulgen.
   B. James Watson.
   C. Erwin Chargoff.
   D. Linus Pauling.
   E. Freidrich Mieschner.

3. The fact that each nitrogenous base in DNA is attached to a molecule of sugar, which is, in turn, attached to a phosphate group to form a single nucleotide was deduced by:
   A. P. A. Levene.
   B. O. T. Avery.
   C. Francis Crick.
   D. Erwin Chargaff.
   E. Linus Pauling.

4. The 46 human chromosomes contain approximately _____ of DNA, which is capable of holding the information in a library of _____ volumes.
   A. one meter; ten
   B. two meters; 1000
   C. 500 meters; 500
   D. one kilometer; 500
   E. two kilometers; 200

5. P. A. Levene made two deductions about the structure of the DNA molecule, the tetranucleotide theory. The correct portion of his theory was that
   A. there are four base units in DNA
   B. DNA is a static, unchanging molecule
   C. the four base units in DNA repeat over and over in the same pattern
   D. DNA is a triple helix

6. During Frederick Griffith's 1928 experiments with pneumococci, _____ were "transformed" into _____ bacteria.
   A. living nonvirulent; living virulent.
   B. living nonvirulent; heat-killed virulent.
   C. heat-killed virulent; living nonvirulent.

D. heat-killed nonvirulent; living nonvirulent.
E. living virulent; heat-killed nonvirulent.

7. Experiments performed in 1943 by Avery, MacLeod, and McCarty indicated that the transforming factor of Griffith's experiments, and therefore the likely genetic material, was
   A. protein
   B. DNA
   C. RNA
   D. a combination of DNA and protein
   E. a combination of DNA and RNA

8. Max Delbruck and Salvador Luria introduced T-even bacteriophages into the study of genetics. These "phages" infect
   A. the bacteria Escherichia coli.
   B. humans.
   C. Drosophila melanogaster, the fruit fly.
   D. Streptococcus pneumoniae, the bacteria causing pneumonia.
   E. any warm-blooded mammal or bird.

9. Hershey and Chase used bacteriophages in which the bacteriophage DNA and protein were labeled as follows:
   A. DNA labeled with ^{35}S; protein labeled with ^{32}P
   B. DNA labeled with ^{14C}; protein labeled with ^{32}P
   C. DNA labeled with ^{32}P; protein labeled with ^{14}C
   D. DNA labeled with ^{14}C; protein labeled with ^{35}S
   E. DNA labeled with ^{32}P; protein labeled with ^{35}S

10. From their experiments Hershey and Chase concluded that DNA rather than protein carries the hereditary message in T-even bacteriophages, because:
    A. offspring virus particles inside the cell contained ^{35}S.
    B. once inside the cell, the protein was inactivated.
    C. the protein doesn't have enough amino acids to carry the needed amount of genetic information.
    D. only DNA was injected into the bacterial cells while the protein coats remained outside the cells.
    E. the new generation of virus particles contained no protein, but did contain DNA.

11. Levene's "tetranucleotide theory" was shown to be incorrect by the work of
    A. Alfred Hershey
    B. Maurice Wilkens
    C. Max Delbruck
    D. Erwin Chargaff
    E. Martha Chase

12. DNA had to possess the capacity for four different activities if it were indeed the genetic material. Which one of the following is **not** one of these four activities?
    A. transformation
    B. replication (duplication)
    C. mutation
    D. chemical stability
    E. information transfer from parent cell to daughter cell

13. The scientist who first suggested that DNA may have a helical structure similar to that of some proteins was
    A. Linus Pauling.
    B. Rosalind Franklin.
    C. Maurice Wilkins.
    D. Erwin Chargoff.
    E. Martha Chase.

14. Which of the following expressions correctly summarizes the findings of Erwin Chargoff?
    A. A = G; T = C
    B. A = T; G = C
    C. A = C; G = T
    D. A = T; G = C
    E. A (not =) T (not =) G (not =) C

15. In the ladder analogy of DNA structure, the subunits which form the upright "rails" of the ladder are held together by
    A. hydrogen bonds between base pairs.
    B. covalent bonds between sugar molecules and nitrogen bases.
    C. covalent bonds between phosphate groups and nitrogen bases.
    D. hydrogen bonds between adjacent nucleotides.
    E. covalent bonds between sugar molecules and phosphate groups.

16. Using the ladder analogy of the structure of DNA, the "rungs" of the ladder are
    A. phosphate groups.
    B. paired nitrogenous bases.
    C. deoxyribose sugar molecules.
    D. hydrogen bonds.
    E. alternating sugar molecules and phosphate groups.

17. DNA has a linear directionality because a phosphate group attaches to the _____ carbon of one sugar and to the _____ carbon of the next sugar in the chain.
    A. 3′; 1′
    B. 1′; 5′

C. 3′; 5′
D. 4′; 5′

18. The nitrogen bases in DNA that contain two nitrogenous rings are
A. the purines adenine and thymine.
B. the pyrimidines guanine and thymine.
C. the pyrimidines ctyosine and adenine.
D. the purines adenine and guanine.
E. the pyrimidines thymine and cytosine.

19. A purine and a pyrimidine that are capable of hydrogen bonding to form the "rung" units of DNA are termed
A. complementary.
B. homologous
C. antiparallel.
D. semiconservative.
E. nonparallel.

20. If you were given the following sequence of nucleotides in DNA:

5′-CATTAGATCG-3′

which of the following would be the correct complementary strand of DNA?
A. 5′-CGATCTAATG-3′
B. 3′-TGCCGAGCTA-5′
C. 3′-GTAATCTAGC-5′
D. 5′-ACGGCTCGAT-3′
E. 3′-CATTAGATCG-5′

21. Since the 5′ to 3′ direction of one strand of the DNA molecule is opposite to that of the other strand, the two strands are said to be
A. complementary.
B. homologous.
C. antiparallel.
D. semiconservative.
E. nonparallel.

22. DNA replication occurs during which part of the cell cycle?
A. G_1 phase
B. S phase
C. G_2 phase
D. prophase
E. meiosis I

23. During replication, each strand acts as a _____ since it serves as a pattern for the formation of a _____ strand.
A. replicate; complementary strand
B. template; identical strand

C. master; double helix
D. blueprint; double helix
E. template; complementary strand

24. In the experiments of Meselson and Stahl to identify the mechanism of DNA replication, the DNA of E. coli was labeled with ^{15}N (heavy nitrogen). The cells were then allowed to undergo one replication in a medium containing ^{14}N (light nitrogen, the most common isotope). When the progeny DNA was isolated and centrifuged in a density gradient, the researchers found
A. a single band of heavy DNA.
B. a single band of light DNA.
C. a single band of DNA intermediate between heavy and light DNA.
D. three bands of DNA, one heavy, one light and one intermediate.

25. The enzymes which catalyze the synthesis of a new DNA strand are called
A. helicases.
B. DNA polymerases.
C. RNA polymerases.
D. topoisomerases.
E. exonucleases.

26. An enzyme that links short segments of DNA together is known as DNA
A. ligase.
B. endonuclease.
C. polymerase.
D. exonuclease.
E. topoisomerase.

27. Watson and Crick, in 1953, speculated that the heredity information is contained in what DNA feature?
A. sequence of nitrogen bases
B. hydrogen bonding between base units
C. alpha helical structure
D. antiparallel nature of the strands
E. phosphate-sugar backbone of the strands

28. The concept of inborn errors of metabolism was first proposed by
A. James Watson
B. Francis Crick
C. George Beadle
D. A. Garrod
E. V. Ingram

29. Beadle and Tatum proposed the "one gene-one enzyme" concept.
In its original form, this hypothesis stated that:
A. one DNA molecule contains the information to make one enzyme.
B. a sequence of nucleotides in DNA contains the information to make one enzyme.
C. each gene has the information to make one lipid and one carbohydrate as well as one enzyme.
D. each gene is actually an enzyme that catalyzes the production of one protein molecule.
E. each polypeptide is the result of the activity of one enzyme.

30. Beadle and Tatum worked with Neurospora crassa and were able to show the relationship between mutations and enzyme pathways. Mutants could be identified by the loss of the ability to manufacture a specific
A. amino acid
B. disaccharide
C. lipid
D. disaccharide
E. amino acid

31. Ribosomes are composed of
A. proteins and RNA.
B. RNA and DNA.
C. DNA and carbohydrates.
D. carbohydrates and lipids.
E. phospholipids and proteins.

32. The sugar in the RNA molecule is _____, while the sugar in the DNA molecule is _____.
A. deoxyribose; ribose
B. fructose; sucrose
C. lactose; deoxylactose
D. ribose; deoxyribose
E. sucrose; fructose

33. The nucleotide _____ is found only in RNA.
A. uracil
B. adenosine
C. guanine
D. cytosine

34. RNA is synthesized on a DNA template in the process called
A. translation.
B. transcription.
C. transformation.
D. transliteration.
E. transmutation.

35. The RNA molecules produced using DNA as a template and which carry the information to code for polypeptides are known as
A. hRNA
B. mRNA
C. rRNA
D. sRNA
E. tRna

36. The RNA molecule produced during transcription is _____ to the DNA template and is synthesized in the _____ direction.
A. parallel; 5′ to 3′
B. parallel; 3′ to 5′
C. antiparallel; 5′ to 3′
D. antiparallel; 3′ to 5′
E. complementary; 3′ to 5′

37. Given the DNA template sequence:

3′-TACATGTTCCAGCCTACT-5′

which of the following would be the complementary mRNA?
A. 5′-ATGTACAAGGTCGGATGA-3′
B. 3′-AGTAGGCTGGAACATAGT-5′
C. 5′-TACATGTTCCAGCCTACT-3′
D. 3′-AGUAGGCUGGAACAUGUA-5′
E. 5′-AUGUACAAGGUCGGAUGA-3′

38. The sequence of three nucleotides in a mRNA molecule that codes for a specific amino acid is the _____, and the complementary sequence on the tRNA molecule is the _____.
A. codon; anticodon
B. promoter; terminator
C. terminator; codon
D. anticodon; promoter
E. anticodon; codon

39. Proteins contain how many different amino acids?
A. 64
B. 40
C. 20
D. 4
E. 2

40. Since there is more than one codon for many of the amino acids, the genetic code is said to be
A. degenerate.
B. regenerate.
C. multifaceted.
D. liberal.
E. conservative.

41. The substitution of one nucleotide for another is known as
    A. insertion
    B. deletion
    C. a point mutation
    D. a frame shift
    E. a somatic mutation

42. Which statement is true of mutations?
    A. mutations involve only changes in the nucleotide sequence of a gene
    B. mutations in body cells are transmitted to future generations
    C. point mutations do not alter the primary structure of a protein
    D. point mutations involve the deletion or addition of nucleotides within a gene
    E. all mutations have an undesirable effect on the organism

43. Once transcription has been completed, which component is **NOT** necessary for protein synthesis to proceed?
    A. mRNA
    B. DNA
    C. ribosomes
    D. tRNA
    E. amino acids

44. A cell has how many different types of transfer RNA molecules?
    A. 5
    B. 10
    C. 15
    D. 20
    E. 25

45. Amino acids are carried to the site of protein synthesis by
    A. n-RNA molecules
    B. m-RNA molecules
    C. ribosomal RNA
    D. DNA molecules
    E. t-RNA molecules

46. The process by which proteins are synthesized is know as
    A. replication
    B. transcription
    C. transformation
    D. translation
    E. conversion

47. In a bacterial cell, a relatively small, self-replicating circular DNA molecule that is separate from the chromosome is a(an)
    A. episome
    B. plasmid
    C. pilus
    D. prophage
    E. capsid

48. Which of the following is not characteristic of plasmids?
    A. they can move into and out of the bacterial chromosome
    B. they are circular and self-replicating
    C. they are equal in size to bacterial chromosomes
    D. they are capable of being cut with restriction enzymes
    E. they are passed from parent bacterial cell to daughter cell

49. Noncoding sequences of a gene are called _____, whereas coding sequences of a gene are called _____.
    A. exons; introns
    B. neutrons; positrons
    C. introns, exons
    D. positrons; neutrons
    E. neutrons; introns

50. Reproduction of new DNA occurs
    A. in the nucleus
    B. within the mitochondrion
    C. at the ribosome
    D. in the cytoplasm
    E. within the Golgi apparatus

51. The inheritance you received from your parents consisted of
    A. physical features of the same-sex parent.
    B. cytoplasmic molecules.
    C. mental attitudes.
    D. instructions for protein synthesis.
    E. RNA molecules.

52. A codon of a mRNA molecule attracts a tRNA molecule with its
    A. DNA
    B. anticodon
    C. peptide bond
    D. code
    E. thymine base unit

53. A DNA segment of base sequences ATAGCATGCACC will probably transcribe how many RNA bases?
    A. one
    B. two
    C. three
    D. six
    E. twelve

54. RNA differs from DNA by each of the following **except**
    A. one of the nucleotide bases
    B. number of strands
    C. the sugar present
    D. being a nucleotide
    E. the proteins in the molecules

55. In the operon, the gene producing the repressor substance is the
    A. operator
    B. promoter
    C. regulator
    D. repressor
    E. corepressor

56. The most accurate hypothesis explaining gene activity is
    A. one gene-one enzyme
    B. one gene-one hemoglobin
    C. one gene-one polypeptide
    D. one gene-one protein
    E. one gene-one DNA molecule

57. The anticodon for codon UCA, red from left to right, is
    A. AGU
    B. ACU
    C. TCU
    D. TGU
    E. TUG

Free Response Questions

I. Discuss the "central dogma of molecular biology." You may use a diagram to help support your discussion but do not draw only a diagram.

II. Given the sense strand of DNA below (and the table of codons provided) predict the anticodons, codons, and the polypeptide chain that would be formed by transcription and translation.

5′ TACGGATTCCACGGTTATATC 3′

III. Discuss the operon theory of genetic control in prokaryotic cells. Use the lac operon as your model.

IV. Discuss different types of mutations and their effects.

APPENDIX F

TABLES

POST TEST ITEM ANALYSIS

Pooled Data of All Classes (n=46)

Number of Incorrect Responses

Item	Incorrect Responses	Item	Incorrect Responses	Item	Incorrect Responses
1	15	20	3	39	13
2	24	21	21	40	33
3	43	22	40	41	30
4	35	23	12	42	43
5	14	24	23	43	8
6	27	25	15	44	23
7	13	26	29	45	17
8	15	27	13	46	14
9	22	28	17	47	10
10	22	29	32	48	18
11	20	30	19	49	23
12	32	31	20	50	8
13	17	32	11	51	8
14	12	33	6	52	29
15	18	34	4	53	17
16	9	35	9	54	28
17	4	36	36	55	17
18	39	37	5	56	21
19	14	38	4	57	8

$\bar{x} = 19$

POST TEST ITEM ANALYSIS

Pooled Data of All Classes (n=46)

Number of Incorrect Responses by Type of Question

Knowledge Questions

Item	Incorrect Responses	Item	Incorrect Responses	Item	Incorrect Responses
1	15	19	14	39	13
2	24	21	21	40	33
3	43	25	15	41	30
5	14	26	29	45	17
6	27	28	17	46	14
7	13	31	20	47	10
8	15	32	11	48	18
9	22	33	6	49	23
12	14	34	4	50	8
13	17	35	9	54	28
15	18	38	4	55	17
17	4			57	8

$\bar{x}$= 17

Comprehension Questions

Item	Incorrect Responses	Item	Incorrect Responses	Item	Incorrect Responses
10	22	22	40	30	19
11	20	23	23	36	36
12	32	24	23	43	8
16	9	27	13	51	8
18	39	29	32	52	29
				56	21

$\bar{x}$= 23

Application Questions

Item	Incorrect Responses	Item	Incorrect Responses	Item	Incorrect Responses
4	35	20	3	37	5
				53	17

$\bar{x}$= 24

Analysis Question

Item 42 Incorrect Responses 43

ANALYSIS OF SCORES

Seventh Period Class

	Pretest	Post Test
number of scores (n)	14	14
mean score ($\bar{x}$)	14.4	32.35
standard deviation (s)	5.15	10.89
standard deviation^2 (s^2)	26.52	120.56
standard error of mean ($s_{\bar{x}}$)	1.37	2.91
standard error of difference ($s_{\bar{x}-\bar{y}}$)	3.24	
difference between means ($\bar{x} - \bar{y}$)	17.95	
degrees of freedom (df)	26	
t	5.54	

Fourth Period Class

	Pretest	Post Test
number of scores (n)	14	14
mean score ($\bar{x}$)	12.2	32
standard deviation (s)	3.76	8.57
standard deviation^2 (s^2)	14.13	73.44
standard error of mean ($s_{\bar{x}}$)	1.00	2.29
standard error of difference ($s_{\bar{x}-\bar{y}}$)	2.03	
difference between means ($\bar{x} - \bar{y}$)	19.80	
degrees of freedom (df)	26	
t	7.92	

ANALYSIS OF SCORES

Third Period Class

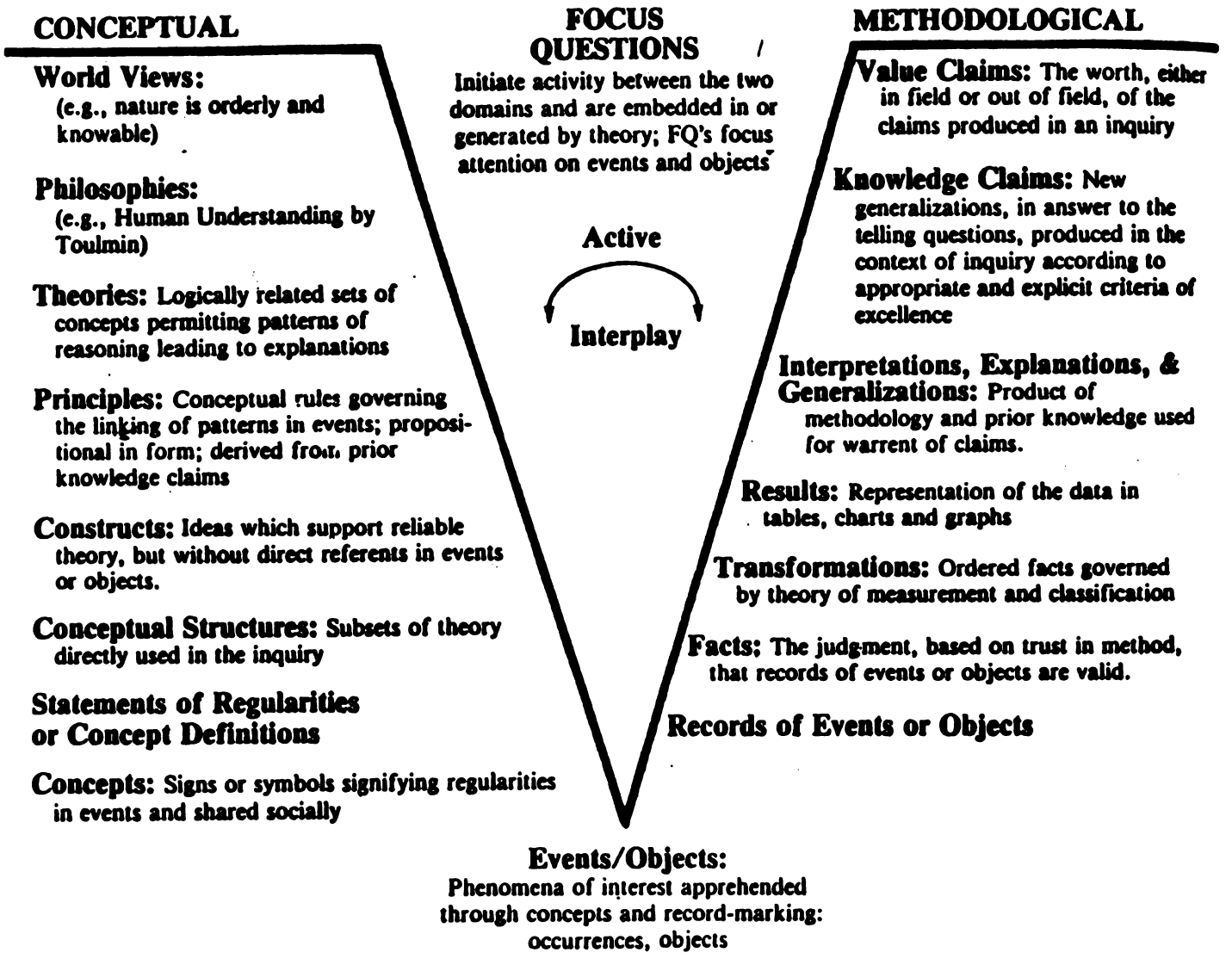
	Pretest	Post Test
number of scores (n)	18	18
mean score (x)	12.27	35.38
standard deviation (s)	3.44	6.76
standard deviation^2 (s^2)	11.83	45.70
standard error of mean ($s_{\bar{x}}$)	0.81	1.59
standard error of difference ($s_{\bar{x}-\bar{y}}$)	1.79	
difference between means ($\bar{x} - \bar{y}$)	23.11	
degrees of freedom (df)	34	
t	12.91	

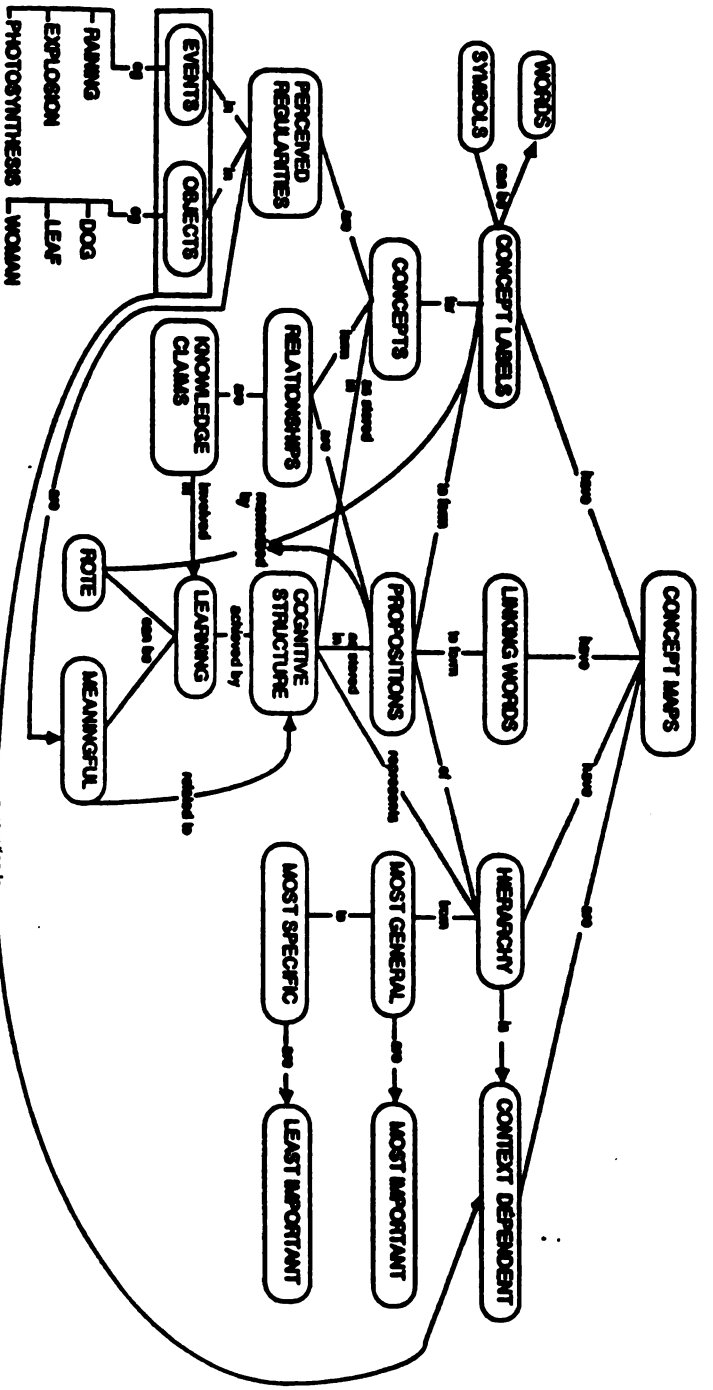
Pooled Data of All Classes

	Pretest	Post Test
number of scores (n)	46	46
mean score ($\bar{x}$)	12.91	33.43
standard deviation (s)	4.15	8.66
standard deviation^2 (s^2)	17.22	75.0
standard error of mean ($s_{\bar{x}}$)	0.61	1.27
standard error of difference ($s_{\bar{x}-\bar{y}}$)	1.12	
difference between means ($\bar{x} - \bar{y}$)	20.52	
degrees of freedom (df)	90	
t	14.55	

APPENDIX G

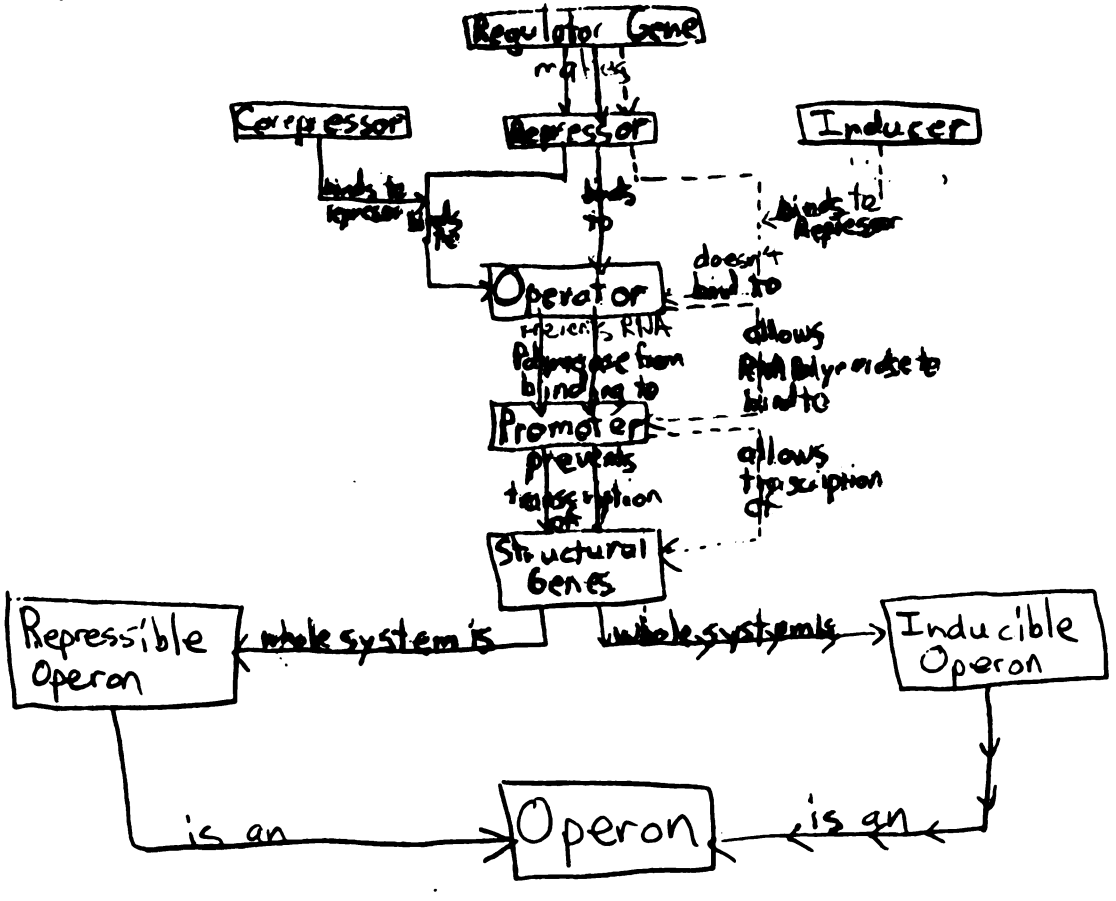
VEE-HEURISTIC AND CONCEPT MAPS


CONCEPTUAL
World Views: (e.g., nature is orderly and knowable)
Philosophies: (e.g., Human Understanding by Toulmin)
Theories: Logically related sets of concepts permitting patterns of reasoning leading to explanations
Principles: Conceptual rules governing the linking of patterns in events; propositional in form; derived from prior knowledge claims
Constructs: Ideas which support reliable theory, but without direct referents in events or objects.
Conceptual Structures: Subsets of theory directly used in the inquiry
Statements of Regularities or Concept Definitions
Concepts: Signs or symbols signifying regularities in events and shared socially
FOCUS QUESTIONS
Initiate activity between the two domains and are embedded in or generated by theory; FQ's focus attention on events and objects
Active
Interplay
METHODOLOGICAL
Value Claims: The worth, either in field or out of field, of the claims produced in an inquiry
Knowledge Claims: New generalizations, in answer to the telling questions, produced in the context of inquiry according to appropriate and explicit criteria of excellence
Interpretations, Explanations, & Generalizations: Product of methodology and prior knowledge used for warrent of claims.
Results: Representation of the data in tables, charts and graphs
Transformations: Ordered facts governed by theory of measurement and classification
Facts: The judgment, based on trust in method, that records of events or objects are valid.
Records of Events or Objects
Events/Objects:
Phenomena of interest apprehended through concepts and record-marking: occurrences, objects

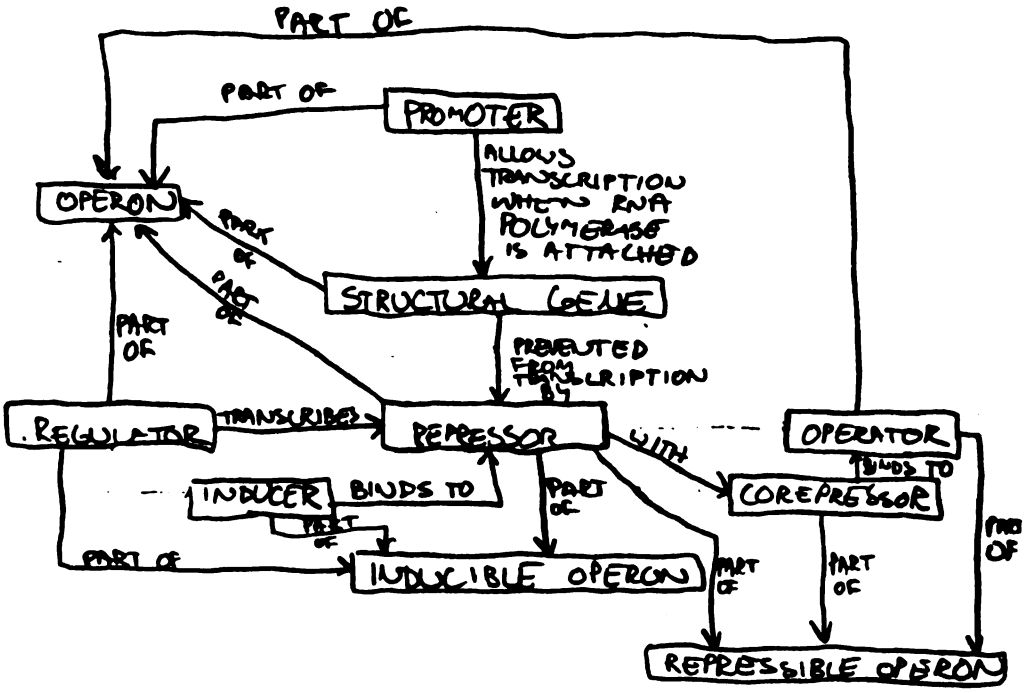


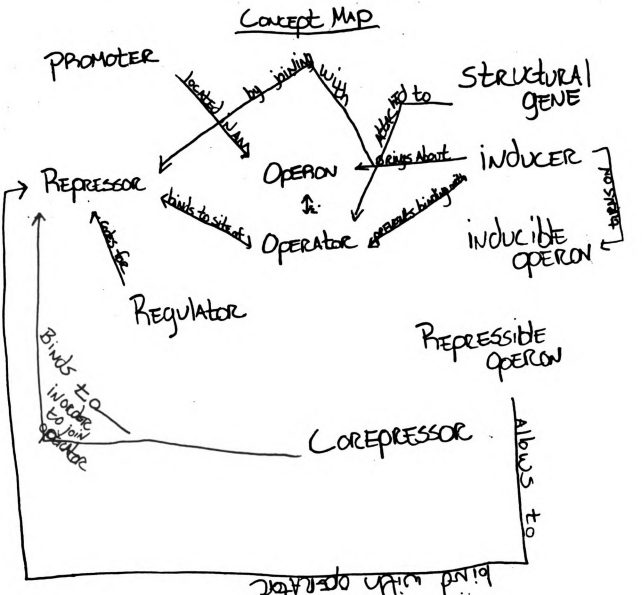
A concept map showing the key features and ideas that underlie concept maps.

Operons-Regulation of Gene Activity Tim Tsey



KEN LENHART


PART OF
PART OF
PROMOTER
ALLOWS TRANSCRIPTION WHEN RNA POLYMERASE IS ATTACHED
OPERON
PART OF
PART OF
STRUCTURAL GENE
PART OF
PREVENTED FROM TRANSCRIPTION BY
REGULATOR
TRANSCRIBES
REPRESSOR
WITH
OPERATOR
BINDS TO
INDUCER
BINDS TO
COREPRESSOR
PART OF
PART OF
PART OF
PART OF
PART OF
INDUCIBLE OPERON
REPRESSIBLE OPERON


Concept Map
PROMOTER
located in/by joining with
STRUCTURAL GENE
REPRESSOR
OPERON
brings about
INDUCER
binds to site of
OPERATOR
INDUCIBLE OPERON
REGULATOR
REPRESSIBLE OPERON
COREPRESSOR
Binds to inactive to join operator
blind with operator

4/13/92

BIBLIOGRAPHY

BIBLIOGRAPHY

Anderson, John N.,1987, "A Basic Laboratoty Course in Modern Biology", Modern Biology Series, Purdue University.

Arms, Karen, and Camp, Pamela S.,1991, Biology A Journey Into Life, Philadelphia: Saunders College Publishing.

Ausuble, David P.,1963, The Psychology of Meaningful Verbal Learning, New York: Grune & Stratton.

Ausuble, David P.,1968, Educational Psychology: A Cognitive View, 2nd edition, New York: Holt, Rinehart and Winston.

Ben-Peretz, M.,1990, The Teacher-Curriculum Encounter: Freeing Teachers from the Tyrrany of Texts, New York: State University of New York Press.

Bloom, Benjamin S.,1956, Taxonomy of Educational Objectives: The Classification of Educational Goals, Handbook I: Cognitive Domain, New York: McKay.

Brooks, J. G. and Brooks, M., 1993, In Search of Understanding: The Case for Constructivist Classrooms, Alexandria, Va.: The Association for Supervision and Curriculum Development.

Champe, Pamela C., and Harvey, Richard A.,1987, Biochemistry, Philadelphia: J. B. Lippincott Company.

Dewey, John,1964, John Dewey on Education, edited by Reginald D. Archambault, Chicago: The University of Chicago Press.

Fisher, K.M.,1983, "Amino Acids and Translation: A Misconception in Biology", In H. Helm and J.D. Novak (Eds.), Proceedings of the International Seminar on Misconceptions in Science and Mathematics (pp. 407-419). Ithaca, NY: Cornell University Department of Education.

Fisher, K.M.,1984, "A Misconception in Biology: Amino Acids and Translation." Davis: University of California Press.

Flanders, M.,1973, "Basic Teaching Skills Derived from a Model of Speaking and Listening." Journal of Teacher

Education, 24,(Spring 73): 24-37.

Fosnot, C.T. (in press), "Rethinking Science Education: A Defense of Piagetian Constructivism." Journal for Research in Science Education quoted in Brooks and Brooks, In Search of Understanding: The Case for The Constructivist Classroom.

Gardner, H.,1991, The Unschooled Mind: How Children Think and How Schools Should Teach, New York: Basic Books.

Goodland, J.,1984, A Place Called School, New York: McGraw-Hill.

Goodman, David S.,1988, Teaching Reasoning Skills in Schools, as quoted in an unpublished thesis by Kelly Cook at John Carroll University, 1993.

Hackling, M.W., and Treagust, D.F., 1982, "What Lower Secondary Schools Students Should Understand About the Mechanism of Inheritance and What They Do Understand Following Instruction", Research In Science Education, 12:78-88.

Hart, Diane,1994, Authentic Assessment, Menlo Park, Calif.: Addison-Wesley Publishing Company.

Hawkins, D.,1980, Critical Barriers to Science Learning Mountain View Center, University of Colorado at Boulder.

Jackson, P. W.,1986, The Practice of Teaching, New York: Teachers College Press.

Jenkins, Christie L., "Recombinant Paper Plasmids", The Science Teacher, 54:4.

Judson, Horace Freeland,1979, The Eighth Day of Creation. New York: Simon and Schuster.

Kaplan, Eugene H.,1983, Problem Solving in Biology, Third Edition, New York: Macmillan Publishing Company, Inc.

Kargbo, D.B., Hobbs, E.D., and Erickson, G.J.,1980, "Children's Beliefs About Inherited Characteristics", Journal of Biological Education, 14:137-146.

Lochhead, J.,1988, "Some Pieces of the Puzzle", Constructivism in the Computer Age, edited by G. Forman and P. Pufall, Hillsdale, N.J.: Lawrence Erlbaum Associates.

Mader, Sylvia S.,1990, Biology Third Edition, Dubuque, Iowa: Wm. C. Brown Publishers.

McClosky, M., A. Caramazza, and B. Green, December 1980,"Curvilinear Motions in the Absence of External Forces:

Naive Beliefs About the Motion of Objects." Science 210:1139-1141.

Mendenhall, William,1979, Introduction to Probability and Statistics: Fifth Edition, North Scituate, Massachusetts, Duxbury Press.

Micklos, David A., and Freyer, Greg,1990, DNA Science: A First Course in Recombinant DNA Technology. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Myers, Richard, 1988, "Recombinant DNA Technology in the High School Biology Laboratory", American Biology Teacher, 50(1):43-45.

Novak, J.D., and Gowin, D. Bob,1984, Learning How to Learn, NeW York: Cambridge University Press.

Piaget, J. and B. Inhelder,1971, Psychology of the Child. New York: Basic Books.

Posner, G.J., Strike, K.A., Hewson, R.W., and Gertzog, W.A., 1982, "Accommodation of A Scientific Conception: Toward a Theory of Conceptual Change." Science Education, 66(2): 211-227.

Smith, E. L.,1990, "Implications of Teachers' Conceptions of Science Teaching and Learning", What Research Says to the Science Teacher Volume 6: The Process of Knowing. Washington, D.C.: National Science Teachers Association.

Smith, M.U.,1989, "Problem Solving in Biology- Focus on Genetics." What Research Says to the Science Teacher Volume 5: Problem Solving. Washington, D.C.: National Science Teachers Association.

Stryer, Lubert,1988, Biochemistry. New York: W. H. Freeman and Company.

Waters, Charles,1992, Statistical Methods for Biology Teachers, Princeton, N.J., The Woodrow Wilson National Fellowship Foundation.