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Christopher P. Forbush

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DNA SCIENCE IN THE HIGH SCHOOL CLASSROOM

By

Christopher P. Forbush

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ABSTRACT

DNA SCIENCE IN THE HIGH SCHOOL CLASSROOM

By

Christopher P. Forbush

As science technology becomes more of a focus in the high school classroom, it was my intention to develop and implement a unit based on DNA science and technology. This unit includes new and exciting strategies that involve students working in groups on problem solving skills, and scientific technical skills within the context of DNA science. I have blended in several situations that the students can appreciate as they take advantage of "real life" situations and problems concerning Recombinant DNA. Assessment is through oral presentations and written work.

Dedicated to Audrey, Samantha and Nicholas

ACKNOWLEDGMENTS

After taking the Cell Molecular Biology for Teachers course at Michigan State University, my mind was made up. At that point in time I realized how important it was for me to develop a unit on DNA science. Taking on this endeavor, was a challenging and satisfying task. I received constant support and motivation from many people.

First, Dr. Merle Heidemann was very supportive throughout the entire process. She has been a constant resource in the classroom, during my research and finally in writing this thesis. The staff at Fowlerville High School has provided me with constant guidance while I worked to improve the unit and to utilize the computer in developing this document. Finally I would like to express my genuine appreciation to my family. My wife and my children provided me with unfailing support, encouragement and patience. THANK YOU.

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INTRODUCTION

It almost didn't happen. In 1951 a young and very shy biochemist, a man who had the ambitions to isolate himself on a wildlife refuge, met an English physicist, who fortunately was a very outgoing person. Within minutes the two were in deep conversation about deoxyribonucleic acid. James Watson, our 24 year old "shy guy" had just started a long term relationship with the scientist Francis Crick. This scientific partnership developed for more than two years as they carried out an intensive and successful study of the DNA molecule at Cambridge University.

It almost didn't happen. Watson and Crick were very close to missing quite possibly one of the world's greatest scientific discoveries. With the help of Rosalind Franklin, a genius of crystallography at King's College in London, they developed a model of the DNA molecule. Many scientists were working on the model at the same time but Watson and Crick were published well before anyone else. I feel it was their diverse personalities that allowed them to talk, exchange, and brainstorm their ideas that finally led to their success. This free exchange of knowledge and information is the very nature of science.

Being able to share and exchange ideas and viewpoints with others is paramount in the field of science. Good communication is one of the most important tools that can be used in this process of figuring out puzzles. Watson and Crick had solved a puzzle that for years had stymied scientists (Jaroff 56). By finally apprehending the double-helix structure of deoxyribonucleic acid, the giant molecule of heredity, they

had paved the way to a leap forward in human understanding of the processes of life (Jaroff 57).

The collaborative effort put forth by these two gentlemen has had an incredible impact on the world. In 1973 the first technique for recombining genes was patented, setting the stage for the biotechnology revolution and the industry built on it.

Since then the field of biotechnology and recombinant DNA has progressed by leaps and bounds. The birth of the first genetic engineering company, Genentech, and the identification of the first cancer causing gene both occurred in 1976. In 1977 a human hormone was successfully produced in bacteria using recombinant DNA techniques. The next year the gene for human insulin was cloned. Next "Genetic Fingerprinting" was introduced and was being used for criminal investigation in Britain during the year of 1985. Five years later the National Center for Human Genome Research was created to lead the U.S. effort in mapping and sequencing the entire set of human genes. Moreover, from 1975 to the present, several genes have been identified including the gene that causes Huntington's Disease.

Since 1952, when Rosalind Franklin first took X-ray diffraction photographs of DNA, to the present, as we are now mapping genes, scientists have made great strides in biotechnology and recombinant DNA procedures. We have come a long way.

Or have we? Has everything been done to keep the ball rolling? Science does not simply consist of doing research and publishing the results. Inspiring and educating young people is the beginning of the

scientific process. If aspiring students are not exposed to current and updated material then this process cannot proceed. As teachers we must instill in our students the passion of finding answers to questions that have not yet been answered.

Hopefully it doesn't just end with a student maturing and logically testing to find answers. Ideally someone, possibly that same student, will now take the responsibility to pass along the new information to another young aspiring student to begin the process again. It is because of this basic idea of trying to motivate students to gain an appreciable interest in the DNA science that I have decided to develop and evaluate a unit on biotechnology and recombinant DNA.

In 1992, the Fowlerville Schools began the time consuming process of becoming accredited by the North Central Association, an organization recognized by universities, that sets curriculum standards in order to improve the success of the students on their proficiency scores. Six target groups were adopted as recommended by the staff to improve areas in the curriculum. One of the groups' jobs was to coordinate a schoolwide strategy to enhance and strengthen the outcome of higher level thinking skills. Through observations made by the teachers, they felt that this was an area that should be emphasized. These are skills that must be used in order for the students to answer scientific questions that they think have no answers.

Another group had the challenge of getting students to use reading and writing strategies across the curriculum. The ability to read and understand content area material is a problem our teachers are faced with

and had to be addressed. Being able to read for understanding and then evaluate and draw conclusions is paramount.

Reading and writing as tools to develop higher level thinking skills can be accompanied by another very helpful process- having students work in groups. Cooperative learning is a great way for all students to get involved in the learning process while they are brainstorming and exchanging ideas. It is unfortunate that evidence shows that science teachers still rely on presenting science by "lecture and answer" techniques (Yager and Penick 51). That's not to say that these techniques do not belong in the classroom, but they should not be the only source for our students to gain an interest in science. A change is needed if we are to keep the ball rolling - rolling in a direction that will inspire students to become critical thinkers, problem solvers and successful scientists.

For all of these reasons: the addition of DNA related labs, an introduction to DNA technology and the implementation of activities to promote higher level thinking skills, my goal was to develop a unit based on DNA science that reflects all of these ideas and strategies. This unit can be used in any high school biology class as it easily fits in any life science and technology curriculum. I implemented this unit in my tenth grade biology class as I have the cooperation of all the other biology teachers in the school. The unit, uses cooperative work and critical thinking skills to introduce students to many facets of DNA science. The science content includes the basic structure and function of DNA, history of the discovery of the molecule, protein synthesis, and an introduction of recombinant DNA and Biotechnology.

COURSE OUTLINE

I. Lecture Topics

- A. DNA structure (Day 1)
  - 1. chemistry
  - 2. bonding
- B. DNA replication (Day 2)
- C. DNA Isolation Techniques (Days 3-5)
- D. Protein synthesis (Days 6-8)
  - 1. transcription
  - 2. translation
- E. Gel electrophoresis (Day 9-11)
- F. Restriction enzyme maps (Day 13-14)
- G. Recombinant DNA (Day 15-17)
- H. PCR (Day18)
- I. DNA Sequencing (Day 18)
- J. DNA fingerprinting (Day 19)

II. Labs and Activities

A. DNA molecule coloring activity (Day 1)

B. DNA replication coloring activity (Day 2)

C. Isolation of DNA from Onion Tissue Lab (Days 3-5)

D. The Size of the Human Genome Activity (Day 5)

E. How Genes Make Proteins Activity (Day 8)

F. Gel Electrophoresis Using Dyes Lab (Days 10 - 11)

G. Palindromes Activity (Day 12)

H. Restriction Enzymes Map Activity (Day 14)

I. Recombinant DNA Assimilation Activity (Day 17)

III. Audio - Visual (Day 20)

A. "The Geometry of Life" From the Infinite Voyage Series by Glencoe (Macmillan/McGraw-Hill)

IV. Evaluations (Day 22)

A. Quizzes

B. Post Test

STUDENT CONCEPTS OF
OF LAB ACTIVITIES

A. DNA Isolation

The students are expected to:

1. know that DNA can be removed from a cell with minimal damage to the polymer
2. know that enzymes, even ones found on your hands, have the ability to cut the long strand of DNA making it difficult to get good results.
3. understand precipitate formation
4. discuss the importance of being able to isolate DNA from cells.

B. How Genes Make Proteins

All students are expected to:

1. list at least five types of proteins.
2. list and describe two major processes of protein synthesis.
3. tell where in the cell protein synthesis occurs.
4. discuss the roles of DNA, mRNA, tRNA, and rRNA
5. explain complementary base pairing.

C. Gel Electrophoresis

The students should be able to:

1. explain the electrophoresis apparatus
2. perform electrophoresis on a series of dyes and interpret the results.
3. discuss the basic principles of the mechanics of gel electrophoresis.

D. Making a Restriction Enzyme Map

All students are expected to:

1. reinforce their understanding of gel electrophoresis
2. explain what restriction enzymes are used for.
3. explain what plasmids are used for.
4. develop a connection between reading an electrophoresis and applying that to where the enzymes made their cleaves.
5. understanding the importance of a restriction enzyme map.

E. Making a Recombinant DNA Plasmid

All students are expected to:

1. explain what restriction enzymes do.
2. explain what plasmids are and what they are used for.
3. understand that DNA is complementary 3' to 5' and 5' to 3'.

4. develop an understanding for the use of restriction enzyme maps.
5. explain what recombinant DNA is and how it can be used.

STUDENT CONCEPTS
WITHOUT LABS

A. DNA Structure and Function

All students are expected to:

1. know the chemical structure of DNA including:
    a. the positions of the sugars, phosphates and bases.
    b. knowledge of the four bases.
    c. understanding of 5' to 3' and its complement strand.
    d. base pairing.
    e. DNA bonding
2. understand the process of DNA replication.

B. Protein synthesis

All students are expected to:

1. understand there is a message contained within the strand of DNA.
2. know the difference between DNA and RNA
3. understand the functions of mRNA, tRNA, and rRNA
4. have an understanding for the process of Transcription.
5. have an understanding for the process of Translation.

C. PCR - Polymerase Chain Reaction

Students should be able to:

1. define PCR and explain the basic outcome of the PCR process.
2. discuss the possible uses of PCR.

D. DNA Sequencing

Students should be able to:

1. define DNA sequencing and explain the basic outcome of DNA sequencing.
2. discuss the possible uses for DNA sequencing.

E. DNA Fingerprinting

All students should be able to:

1. Define DNA Fingerprinting and explain the process of how a fingerprint is obtained.
2. read a DNA fingerprint to make a comparison between individuals.
3. explain some of the uses for a DNA fingerprint.

IMPLEMENTATION OF THE UNIT

With the added emphasis in our curriculum to be hands on and technology oriented, this unit had to be developed by me as most textbooks do not feature biotechnology activities. Although there are many activities that can be found that illustrate DNA biotechnology, a teacher has to dig and search to find them. When they do find them, the activities simply use too many expensive chemicals or require lab equipment that is unavailable. My intention was to develop a unit that will introduce the students to the DNA molecule and then take them a step further by showing them the many things now being done with the polymer, things that may someday have an impact on their lives in the future, if not already.

Students are constantly being exposed to news from the media that in someway may be connected to DNA biotechnology, such as gene splicing, DNA fingerprinting or even cloning. They should have some knowledge of this science as it makes them more qualified to make a judgment one way or another. It is unfortunate when a person is asked to make a decision on an issue that they are unfamiliar with what may very well affect their life or possibly someone else's life.

Take for example the recent O.J. Simpson Murder Trial. The jury had no knowledge of DNA, let alone DNA fingerprinting. When the defense finished their case, many people believed that the jury was so confused that they attributed no validity to DNA science and they simply ignored very incriminating evidence. Or, as an analogy, in every

classroom there is a student that is a diabetic and as for the rest of the class, they usually know someone who is. However, if you ask the class where insulin comes from, they haven't a clue. This is by no means the fault of the students, they have just never been exposed to concepts about Recombinant DNA.

The labs and activities that I have incorporated into this unit explain the previously described concepts about DNA and more. When a student has successfully completed this unit they will be able to talk about DNA biotechnology with accuracy and relate what they know with the news they hear from the media and make sound connections.

Most of the labs that are in this unit are simulations that are quite effective, yet they are affordable and easy for a teacher to prepare. Some of the labs were pulled from various sources while others I wrote as a result of watching a number of students struggle with these topics.

In addition to the labs and activities, I have incorporated some lectures, films, role playing activities and some drawings that the student can get involved with. The unit requires about 4 weeks to complete. For the two years that I have taught the unit, I tried pretesting the students before the unit and found that they had no prior knowledge of this material as they simply left their pretests blank. Upon completion of the unit the students were tested to assess the success of the unit. Also, I exercised the option of interviewing some students before and after the lessons either verbally or in writing. The basic requirements for the unit consist of a student generated folder, quizzes, labs and activities, attendance, participation and a final test.

The point values I assign are listed below:

Attendance and participation	100 points
Folder	100 points
Quizzes	50 points
Labs and activities writeups	150 points
Post test	100 points

There are a total of 500 points that can be earned in the unit. As can be seen the post test only counts for only one fifth of the total unit points. I was more concerned that the student be exposed to as much DNA biotechnology as possible without being burdened by simply recalling facts. To begin, attendance was very important as the concepts that are being taught are usually group driven. When the students were in their groups after each lecture, they have to use a brainstorming process, much as Watson and Crick used, to obtain a thorough understanding of the concepts. Thus, it is imperative that students be present and actively participating within their group. To facilitate this activity, the instructor can simply walk around the room and monitor the groups and when they are done, have a person in each group report on their progress or findings.

Next, the students were required to accumulate their work in a folder that may be used at some later date. The labs, activities, quizzes and tests will be an excellent source of information for them to use as they finish the rest of the class and also when they move on to college.

The quizzes were used to reinforcement the lectures and lab activities. A total of five quizzes were given.

A list of the quiz topics are below:

Quiz #1	DNA structure and isolation techniques
#2	Protein synthesis
#3	Gel electrophoresis
#4	Restriction enzyme maps
#5	Recombinant DNA

Lab activities are worth the most points as they demonstrate that the students are brainstorming within their group. These groups prove to be a good learning process as they are learning to work with others and master a concept. The students were in their "teacher assigned" groups whenever we did a lab. They consisted of two to three students per group. When the lab or activity was finished, the students wrote a short synopsis of what they accomplished to help reinforce what they have just learned along with practicing their writing skills.

EVALUATION

This unit that I prepared is currently being used at our high school by all 4 of the biology teachers. It was my intention as well as that of the other teachers to bring the students up to date on current issues in DNA science and involve them in some activities that are actually being used in biotechnology.

The material in this unit does lend itself to pre and post tests, but this is actually the first time that most of the students were exposed to the topic of DNA. Therefore, a traditional pre test was not extremely useful. As I indicated before, most of them knew very little about DNA science. However, I did have them fill out a questionnaire that asked them to rank their understandings of some topics in DNA science, but again they did not rank their knowledge very high. The results to this questionnaire will be discussed later in this section.

Next, I tried to develop the unit in a fashion that caught the students' attention and maintained a high interest level. This is no easy task as 10th graders seem to lose interest in a hurry. By using a combination of labs, minimal lectures, cut and paste activities and lots of enthusiasm, I was able to have an impact on the students' interest throughout most of the unit. By listening to some of the comments made by the students in the post interviews, such as "this was a fun and interesting chapter", the students maintained an interest level that I perceived as an improvement over most topics. There were many more

areas of the unit wherein the students expressed a great deal of interest and enjoyment.

The atmosphere of the class was informal as most of the activities were group oriented. Instructive lectures, which I tried to keep as short as possible, were very structured and to the point. I never lectured for more than 20 or 30 minutes at one time. Brainstorming was emphasized during this time, as I felt it was important to involve them as much as possible in the learning process. After each lecture was complete, I always provided them with an activity or a few short questions that pertained to the lecture. For example, after the lecture on PCR, I had the students get into groups of two or three and develop a definition and a list of some uses for PCR.

Although most of the students were quite interested in the topics each day, a fair amount of structure was still required as the students needed some deadlines for their activities. For example, if we did a lab on Monday, I gave them a short prelab and they began the lab. I allowed enough time to finish the lab, about one or two days, but immediately following, they had to turn in a written summary of what they learned during the lab. This seemed to reinforce the concepts being taught in the activity.

I do feel it is important to mention that the instructor should assign the students in their particular groups while they are working on their lab activities. I traditionally put an A student, a B student and a C student in each, trying to maintain a range of abilities in each group and separating "best friends". Each student in the group was required to turn in a lab report and a summary. To emphasize the importance of a good summary,

I allowed the students to use them during their quizzes. However, they did not use their own summary, but rather that of their lab partners. This forced them to make sure everyone in the group understands the concepts being learned. I noticed a trend in the quiz grades, as they steadily improved throughout the unit. The grades only improved slightly (less than 5%) but the material did get more difficult as the unit moved on. This slight increase may have been attributed to their summaries but one thing was for sure: I definitely observed an appreciable change in attitude. An attitude that reflected a concern for everyone in the group to provide a good summary.

Many comments were made by my students during the Post-interviews that reflected the strengths and weaknesses of the unit. Twelve students, every tenth one in the alphabet, were interviewed. Their grades ranged from A's to D's as I tried to get a random sample. The sample of students were asked six questions that pertained to the unit. The questions were as follows:

1. How would you compare this unit with other units regarding:
    a. the content
    b. the labs
    c. usefulness
    d. up to date material

2. Was the material in this unit:

a. too difficult to understand?

b. too easy to understand?

c. at about the right level?

3. What was the most interesting part of the unit?

The most repeated comment, regarding the content, labs and the usefulness of the unit, made by almost one half of the students was "everyone should have to learn about DNA science, even adults". This comment intrigued me so I reinterviewed those students to see what they meant. The majority of them were referring to the fact that adults form ideas and make decisions about DNA science on juries, during paternity suits, and even when talking to their doctor about someone's health, including their own. It seems as if students didn't think that the adults had much faith in DNA science. I also was intrigued to find that 11 of the 12 students said that the material was taught at the appropriate level.

Other comments included:

"I enjoyed this unit because it was very interesting."

"The labs were fun and interesting."

"This chapter was interesting because now I know about DNA and how it is different and also the same in different people."

"I liked working in groups and doing the labs."

"I still can't believe how much information is in one cell. The "size of the genome" lab was cool."

Before starting the unit I had the students complete a questionnaire on several topics related to DNA science. I had all of my students take part in this as I only had 105 tenth graders involved. I began by asking them if they had any prior knowledge of ten different topics. When the unit was over I gave them the same blank questionnaire and asked them about their knowledge of these topics again. They rated their knowledge on a scale of 0 - 5 with 0 meaning they had no knowledge of the subject and 5 representing they knew a lot about the subject. The results are provided on the next page:

TABLE 1 - Percent Change in Student Knowledge of Different Unit Topics Learned

Before	Topic Learned	After	Percent Change
2.03	DNA Structure	4.61	127
2.11	DNA Function	4.33	105
1.87	Relative Size OF DNA	3.83	104
0.84	Protein Synthesis	4.14	393
0.61	Electrophoresis	3.57	485
0.43	Restriction Enzyme Map	2.81	553
0.84	Recombinant DNA	3.24	286
0.25	PCR	2.59	936
0.39	DNA Sequencing	2.17	456
1.86	DNA Fingerprinting	3.88	108

This table demonstrates the students' knowledge of each topic before and after being taught the unit. A scale of 0 - 5 was utilized (5 = great knowledge and 0 = no knowledge). Data was obtained from a sample of 105 students in the Fall of 1995 and 1996.

TABLE 2 - Ranking The Percent Change From Table 1

Topic	Percent Changed
PCR	936
Restriction Enzymes	553
Electrophoresis	485
DNA Sequencing	456
Protein Synthesis	393
Recombinant DNA	286
DNA Structure	127
DNA Fingerprinting	108
Realative Size of DNA	105
DNA Function	104

Table 2 ranks the percent change in knowledge of each topic after teaching the unit in order of the most to least change.

From the information on this pre-unit questionnaire I assume that the students have had some knowledge of DNA structure and function and some exposure to DNA fingerprinting. Our 8th grade program does teach about the cell and a little about DNA, but their introduction to fingerprinting came through the media or other reading.

A Large percent change in self-reported knowledge of the other six area shows that the students had hardly any prior exposure to these particular subjects. PCR had the largest jump in knowledge gained as it was over 900%. Restriction enzyme mapping, electrophoresis, and DNA sequencing concepts also had a significant increase in knowledge as they ranged from a 400 to a 500% gain.

After this questionnaire, students answered some written questions from a pre test which was eventually followed by a post test. The pre test can be found in appendix III. The purpose of the pretest was to have the students write as much as possible about their knowledge of DNA without the worry of the answers counting against them.

Six post-test questions were targeted to see how the students' response changed after they had been taught the unit. The first question was: "what is DNA's basic shape and where is it found?" For the first part of the question more than 70% of the students answered: cells, genes, and chromosomes. Only about 2-4% could actually say anything about the fact that it is a long polymeric molecule that exists like a twisting ladder. It seems as though they have not made the relationships between DNA, the cell and genes. Although they received the highest score for this question in their interview about DNA structure , I think it would be safe to

assume that they were not as knowledgeable about this question as I thought.

In response to the second half of the question "where can DNA be found", the most prevalent answer made by 48% of the students was "only in the blood". This seems to make a reference that they are watching the news and listening to stories that make references to DNA testing in crimes.

The next question that I targeted was: "is it possible to remove DNA from a cell without damaging the molecule"? The majority of the students (53%) thought that this procedure was possible and 41% believed that it could not be done. I thought this statistic was interesting, because I posed another question: "is it possible for a scientist to compare the DNA of one individual with another" and I found that 96% said that this procedure is possible. It is interesting that they would think that DNA could be compared but it could not be removed from a cell.

Another question I posed was:"is it possible for two people to have the same copy of DNA"? The results were not surprising as 92% of the 105 surveyed said "no" but only 13 % went on to mention anything about twins, triplets etc. I followed up this question with: "does your liver cell have the same copy of DNA as one of your heart cells"? It was interesting to see that only 43% said yes and 57% responded no.

The post test results were very positive as the students seem to have grasped the material. To start, about 94% of the students knew the chemical make up of DNA as they could explain the shape or draw the molecule and 100% could tell you that it was found in the nucleus of all

cells. Continuing with the question: "is it possible to remove DNA from a cell without damaging it", the results were very reassuring. The "DNA isolation from onion tissue lab" must have made a strong impact as 94% of them responded that it is possible. Also 99% of them learned that scientists can compare DNA from one individual to the next.

Turning to the question: "is it possible for two people to have the same DNA", the results didn't change very much as they seem to be quite knowledgeable before the unit, but they did respond more often about twins. The "no" response increased from 92% to 94% but their inclusion of twins went from 13% to 65%.

A significant increase in correct responses was found on the question: "does your liver cell have the same copy of DNA as one of your muscle cells". A "yes" response was increased from 43% on the pre test to 92% on the post test.

I felt that the results of the post test were encouraging as all of the target questions reported a significant increase in correct responses. Obviously after teaching the unit, one should see an increase in correct responses, however I feel these increases were significant because the target questions were concept-oriented and not just recalling facts.

At my high school there are seven sections of biology, each class averaging about 27 sophomores. Each year on average 3 instructors teach these seven classes. Up to this point four teachers, including myself, have had the opportunity to use the unit. Two of the teachers have taught less than five years, while the other teacher is quite experienced

as he has taught for about 16 years. I informally surveyed these three teachers after they used the DNA unit for one year.

The first question I asked was "in teaching this unit, was it a learning process for you as well as the students"? All of the teachers responded with a resounding "yes". One teacher exclaimed,"The unit on DNA biotechnology was a learning process for me. Seven years ago, the last time I taught biology, DNA technology was not a topic I covered in class. This technology was just something I read about in journals. Now, after teaching this unit, I have a greater understanding of the topic".

Another question posed was "do you think the unit was too lengthy"? All of the teachers did think the unit was lengthy but still too important to skip. They commented on how difficult it is to cover everything in our curriculum, yet they still felt comfortable the time required to teach this unit. In fact, most of them felt it should become part of the curriculum.

When asked "do you feel that the labs and activities fostered higher level thinking skills", all of the teachers praised at least two to three activities or labs. The Plasmid Lab, Human Genome Activity and Gel Electrophoresis Lab were mentioned the most. All three of the teachers said that they will use the unit again next year as they thought it was well worth their time.

The last question was, do they feel that it was important for teachers to remain as current as possible in terms of teaching strategies and content information. Obviously they all made it quite clear that it is

very important for us to keep updated on science issues but, it is time consuming and difficult to do in the "information age" that we now live in.

DISCUSSIONS AND CONCLUSIONS

COURSE CONTENT

The unit has changed quite drastically in its development over the past 6 years. To begin, I have replaced many of the labs and activities with other labs that I perceived to be more effective. I started adding these labs in the fall of 1991 after I took the "Cell Molecular Biology" course. Some of these were generated by myself and the rest were written by other teachers. For two years I was able to see what worked and what didn't work in the classroom. When I did my research at MSU in the summer of 1993, I was able to analyze, change and develop more effective labs.

One of the labs I originally tried to use in 1992 was a DNA Electrophoresis Lab. I eventually omitted this lab as we never seemed to get results that the students could determine. In addition it was quite costly, and the advanced students in Forensics and our A.P. biology also do this lab. Further, we did get very good results from the electrophoresis lab using dyes, so I felt that the students exposure to the concept of electrophoresis was sufficient.

Other labs that were removed seemed redundant or overemphasized the ideas being presented. Reinforcement is an effective way to teach, but you have to know when enough is enough. For example, if you are pressed for time, omitting one of the restriction enzyme map labs will work even though they do seem to complement each other.

There are several labs that I perceive as especially effective in conveying a concept. To begin, the "isolation of DNA from onion tissue" is an excellent lab that shows the students the relatively easy way DNA can be removed from a cell without damaging the polymer: although some students did see that it is possible to damage the polymer by producing a fuzzy mass. This activity was prepared by trying to keep costs down and the availability of equipment. Most labs like this use salmon sperm, but this uses something that is easy and cheap to obtain: onions. You get just as good results by producing ample amounts of DNA as long as the students carefully follow directions.

Another lab that works well and is well worth the time is the gel electrophoresis of dyes lab. Although you do need a power source for running the gels, the results are excellent. The students learn all of the principles used in electrophoresis and gain some insight in how molecules can be separated by size. The lab is much cheaper than using DNA and you don't have to use restriction enzymes, markers or dyes. Although I feel it is important for students to run a DNA gel at some point in time, they usually get that chance in a more advanced class as upperclassmen.

In any event, students should be engaged in some type of electrophoresis activity as this is a technique behind DNA science that is usually ignored by most teachers. Although it is an important activity, many classrooms simply do not have the resources. However, I perceive this as a very effective lab. The students enjoyed it as they really feel like they are being scientists by using sophisticated equipment and not just

scissors and paper. In addition to this, the students really appreciate being given the opportunity to do something that is technically difficult and usually only done by adults.

Along with completing the labs and activities, I found that it is well worth the time to have the students summarize each activity when they are done. They were instructed to write the summary in a way so that if they were to give the summary to a person that did not do the lab, that person would fully understand the procedures. The summary should include procedures, conclusions and the concepts being taught. This simple assignment reinforces the understanding of the lab. The students don't like writing these summaries, but I feel that it is an extremely beneficial exercise.

DISCUSSIONS AND CONCLUSIONS
LOOKING TO THE FUTURE

Developing this unit has inspired me to teach my students about DNA science more than I ever imagined. It seems the more you learn about a subject, the more you want others to learn. I never imagined that I could offer extra labs after school for students that are interested in DNA science and actually have a few show up, simply to run DNA gels after we isolated it from onions. In fact, just this year the Michigan Science Olympiad Competition has just added a competition on DNA science, particularly recombinant DNA and DNA fingerprinting. This is exciting for students when they say : "hey, we did this in my biology class", particularly when they find that their competitors have no previous background in this discipline.

Most of today's students are faced with many obstacles while they are maturing. If we can be of any help to them, it is our duty to do so, whether it happens to be teaching them about DNA or helping them adjust to peer pressure. We must look to the future. We must teach current issues and ask students what they think may happen in the future. The problems facing all of us are ones that only those involved in schools can fully appreciate. Teaching is very demanding. In truth, it is difficult to remain current with new methods and concepts to teach our students. Yet, this is exactly what we must do. We look at the students needs and knowledge and develop the course of action accordingly. As teachers, we cannot take the easy way out and simply teach facts. We need to get

students ready for the twenty-first century. To do this our plan of action may include adopting specific attitudes and actions.

For example, students need to see the importance of the education that they are receiving. If the students see the excitement in the activities, and labs displayed by the instructor, they can adopt a positive attitude toward lifelong learning. Growth and change require an open mind. If we can instill the idea that only through new learning, they will see opportunity. Therefore, we, as great teachers, must remain open minded about new strategies and technologies and be ready to use them in class. Remember, they won't be successful in the future unless we are.

SCIENCE BACKGROUND

LECTURE MATERIAL

Structure of DNA

Many techniques are utilized to educate students in DNA science. Initially, the students need to become familiar with the molecule and its discovery. All high school biology text books seem to adequately cover that issue. Most texts address DNA replication and protein synthesis. However, until recently, recombinant DNA and biotechnology were never mentioned. As James Watson and Francis Crick described in March of 1953, the DNA molecule is a ladder of subunits that seems to twist like a spiral, commonly referred to as double helix. The sides of the double stranded polymer are alternating subunits of deoxyribose sugars (pentose, a 5 carbon sugar) and a phosphate group. The subunits that connect the two sides together, or the "rungs of the ladder", are nitrogenous bases. These bases are responsible for genetic variability. Although there are only four different bases, it is the exact order of those bases that makes the difference between a cat and a dog. These compounds are commonly referred to as adenine (A), guanine (G), cytosine (C), and thymine (T). Adenine and guanine are double ring bases named purines. Pyrimidines, thymine and cytosine, are smaller, single ring bases.

A nucleotide is a combination of a sugar, phosphate group, and any one of the four bases. The sugar molecule is the link between the

phosphate and the base. Whether the sugar is ribose as in ribonucleic acid or deoxyribose, in purines the 9 nitrogen atom and in pyrimidines the 1 nitrogen atom are both bonded to the 1' carbon of the sugar. As these nucleotides polymerize to make long strands of DNA, the hydroxyl group or the 3' carbon of the sugar forms an ester bond to the phosphate group of another nucleotide. The formation of the ester bond releases one water molecule. The "tie" between the nucleotides are called phosphodiester bonds.

The rungs of the ladder, the bases, have a sequence that is quite different in every organism. However the bases pair up in a very organized way. The purines always hydrogen bond with the pyrimidines such that adenine (A) only pairs with thymine (T) and guanine (G) with cytosine (C). C is linked to G with three hydrogen bonds and A to T using only two bonds. Theoretically a purine like adenine could form a bond with the pyrimidine cytosine but the helix would become distorted and ultimately the polymer would break down.

Replication of DNA

There are many mechanisms involved in passing along genetic information from one cell to the next. During somatic cell division this mechanism is mitosis. Therefore, there must be a way of making duplicate copies of DNA. The DNA nucleotide sequence in every organism was obtained from its parents. This process of copying DNA is called replication. Keep in mind, if a cell has a genome of approximately 3 billion base pairs then the resultant new cell must have the same 3 billion base pairs in the exact same order. Such duplication is an absolutely amazing process. For something to be this fast and accurate is truly a wonder.

Remember that a DNA molecule is made of two strands, both containing a sequence of nucleotides. Adenine always pairs with thymine and cytosine with guanine. Therefore, if you know the order of the bases on one strand, you can predict the order of the bases on the complementary strand. DNA replication is accomplished in this manner. To begin, the DNA molecule separates as enzymes break the hydrogen bonds between the bases. At this point there are two free strands. Short fragments of single stranded DNA called Okazaki fragments are now being connected to the free strands from the 5' end to the 3' end. The 5' and 3' terms are referring to the carbon atom found on the sugar ring. This is the "leading strand". The other side of the "unzipped" DNA molecule also fills in the missing nucleotides from the 5' to the 3' direction but it is actually in the opposite direction as the first. This is known as the "lagging strand". These free floating nucleotides are attached to the

sugars by using enzymes called DNA ligases. The process continues until the entire molecule has been replicated. As a result, both new strands are an exact copy of the original.

At this point, all of the DNA has been replicated in the cell. When there are two copies of the genome in the cell, the two polymers can be separated and passed on to the new cell through mitosis.

From DNA to Protein

The message or "code" that is dictated by the nucleotide sequences in a strand of DNA is ultimately responsible for the characteristics found in all organisms. How does this code convey its message to carry out these characteristics? The answer lies in the proteins. Proteins are complex polymers of amino acids, that determine structure and function of an organism. Some proteins are enzymes that control chemical reactions and others build and repair cell structures.

Short sections of DNA, called genes, are codes for certain proteins. A human cell may contain up to 100,000 genes. We have long known that genes determine the sequence of amino acids to form a protein for carrying out specific instructions. These instructions, through a multitude of proteins, establish the traits found in all organisms. Thus, the sequence of nucleotides in the DNA is responsible for building proteins.

Like DNA, ribonucleic acid (RNA) is a nucleic acid but it differs from DNA in a few ways. To begin with, RNA is usually a single strand of nucleotides as opposed to the double stranded DNA. Further, RNA also contains four bases like DNA, but RNA has replaced thymine with a base called uracil. Lastly, RNA has a different sugar. RNA contains a ribose sugar and DNA bears a deoxyribose sugar. The reasons for mentioning RNA will become quite apparent as we proceed. It should be made clear at this point in time that there are three types of RNA which are differentiated by their role in protein synthesis.

We know that RNA is the transmitter of information from the nucleus out to the cytoplasm, specifically to the ribosome. To start the process of protein synthesis, a template of RNA from DNA is needed to carry the code out of the nucleus. This process is referred to as transcription. In transcription, the DNA helix molecule unzips with the aid of polymerase enzymes, much like replication, and a single stranded polymer of RNA is made. Unlike the double stranded DNA, the single stranded RNA has one base that is different. Uracil has replaced thymine. The new RNA molecule is called messenger RNA (mRNA). It is appropriately named because it transmits the code out of the cell. Ultimately mRNA holds and carries the information to the ribosome for the making of proteins.

Along with mRNA, the synthesis of proteins require two more types of RNA, a supply of amino acids, a number of enzymes, and ATP as a source of energy. Now that the mRNA has transported the information to the ribosome, we address the ribosomes themselves. The ribosome is about half RNA and half protein. The form of RNA the ribosome contains is called ribosomal RNA (rRNA). Each ribosome is composed of two subunits, each with its characteristic RNAs and proteins. Now that the mRNA is in the cytoplasm it will attach to a ribosome. The mRNA fits between the two protein subunits of the ribosome. The ribosome moves along the mRNA molecule much like a railroad car moves on its tracks. As the ribosome moves along, the codons of the mRNA are exposed at the surface of the ribosome. Codons are a basic unit of the genetic code.

They are simply three adjacent nucleotides in the strand of the mRNA that code for an amino acid.

As each codon is exposed, a molecule of transfer RNA with its particular amino acid attached approaches. Transfer RNA (tRNA) is the third type of RNA that is involved with translation. If proteins are to be synthesized, amino acids must be carried to the ribosome. This process of the tRNA moving the 20 different kinds of amino acids towards the ribosome and then having them attach in the specific order as designated by the mRNA is called translation. Translation is the actual process of bonding amino acids in a specific sequence to form a protein. At this point, each amino acid has an anticodon. The anticodon is a nucleotide triplet that will match the codon. When the anticodons that are being carried by the tRNA assemble on the surface of the ribosome, they will be in a specific order. This stage in the biosynthesis of proteins , the assembly of amino acids in a specific sequence, is translation. The information *transcribed* from the nucleotide sequence of DNA to the nucleotide sequence of mRNA has been *translated* into the amino acid sequence of the protein.

Recombinant DNA Technology

Recently, DNA has been a very hot issue in the media. (Take the O.J. Simpson trial, for example). When DNA is mentioned, the media assumes everyone knows its structure as they are talking about very new and current issues and technologies, such as DNA fingerprinting, cloning, and genetic engineering. To obtain a better understanding of these advances, the viewer should be aware of some basic concepts about the genetic material and related terminology. To begin, the gene is probably the most visible term used. Since the discovery of genes, about 40 years has past and we have a better understanding of how heredity works. Genes, which are sections of DNA that code for a specific trait, can be manipulated by using new techniques that are easy to understand. Keep in mind that recombinant DNA is DNA made by connecting fragments of DNA from different sources. Thus it is easy to see that DNA requires some cutting or "cleaving". Restriction enzymes can be used for this procedure. There are hundreds of these enzymes, all of which have the ability to cleave DNA into short fragments at specific sites that it recognizes. Recall that the nucleus contains about 3 billion base pairs. With this much genetic material it makes it difficult to make cuts in the DNA without getting millions of fragments of DNA. Using these restriction enzymes, the researcher has the ability to make very specific cuts in the DNA, that cleave with unbelievable accuracy. The enzymes recognize a certain base pairs sequence and cut that one sequence throughout the strand of DNA.

After the desired sections of DNA have been cleaved, they can be removed from the cell and inserted into a new host cell. In order to produce recombinant DNA, the cleaved fragment of DNA must recombine with something else. That something else is a vector. A vector is a means by which foreign DNA can be transferred into the host cell. In order to insert this new strand of cleaved DNA carried by a vector, the recipient cell needs to have those same cuts made in its DNA by the same restriction enzymes. Sections of DNA are removed from its genome to make room for the new sections of DNA. This procedure actually leaves two ends to the DNA, commonly referred to as "sticky ends". The nickname is very appropriate because the ends readily attach to the new strands of DNA.

The actual insertion of the new DNA plasmid into the bacteria is done quite easily. Most often, the new DNA strands can just be mixed in with millions of the host cells, usually bacteria, that are suspended in a salt solution. Within a few minutes, several bacteria will take up the new DNA contained in the vector plasmid. At this point, the bacteria, now containing the vector, can be grown in large quantities. The technical term for a large number of cells grown from a single cell is a clone. Therefore, this technique is sometimes known as DNA cloning.

DNA sequencing, PCR and Gel Electrophoresis

Sequencing is determining the order of nucleotide bases along a length of DNA. Only one of the two strands of the double helix is used. However, many copies of this strand are required. Through the use of PCR, polymerase chain reaction, many copies of a DNA strand of interest can be made.

PCR is basically an in vitro DNA replication process. Starting with the original strand of DNA, some primers, a heat resistant DNA polymerase to extend the primers, and a series of heating and cooling cycles, the original DNA segment can be amplified a millionfold in just twelve hours. The procedure is extremely useful when trying to sequence DNA when only very small samples are available. This is commonly used in a murder case when the blood, the source of DNA is found in trace amounts.

The many pieces of this single stranded DNA can be sequenced to find the exact order of the nucleotide bases. There are several methods that can be used. One of the easiest to understand is the Maxam-Gilbert method. In this method, a radioactive label is attached to the 5' end of each strand of DNA. The strands are put into four separate test tubes and subjected to different chemical treatment. These chemicals selectively cut the strand between one of the bases and the rest of the strand. For example, one of the test tubes may contain a chemical that cuts after the base guanine. Therefore, these strands are cut at varying lengths as all of

the cuts are after guanine and the rest of the strand. Some of the strands will include the radioactive marker.

The DNA fragments can now be loaded into a properly prepared gel and then subjected to an electric field. This process, gel electrophoresis, is used to separate fragments of DNA according to their size. The electrophoresis chamber is connected to a power source and the fragments are attracted to either the anode or the cathode. As the DNA fragments travel through the gel, they move at different speeds. The smaller the fragments are, the faster they travel through the gel. After awhile, the DNA radioactive markers show up in the gel. A pattern of bands is revealed. The pattern of bands unveils the order of the bases in the original strand.

DNA Fingerprinting

In 1983, a 15 year old girl was killed in Narborough England. Lynda Mann's body was found raped and strangled on a dark lane near the grounds of a mental hospital. Police scoured the villages for three years without a lead. Then, on a path not far from where Lynda was killed, 15 year old Dawn Ashworth was found. She was also raped and strangled. Within a week, a kitchen worker at the mental hospital confessed to Dawn's murder. Investigators were anxious to find out if the murders were related. Dr. Alex Jeffreys was called in to assist. Alex had developed a test to reveal markers for inherited diseases that may possibly lead to early treatment.

The kitchen worker's DNA from his blood was tested using a technique that Jefferys developed and then compared with the semen recovered from Lynda's and Dawn's bodies. The results were very surprising. It was found that the semen from both bodies were the same, but neither matched the worker from the mental hospital.

At this point, the police launched a huge effort to draw the blood of more than 4,000 young men in the area. This voluntary blood sampling went on for eight months when finally an arrest was made. Colin Pitchfork ultimately confessed to both murders. DNA fingerprinting was the key in this case solving the crime. In short DNA fingerprinting is the process of extracting DNA from a biological sample such as blood semen etc, and then is subjected by restriction enzymes that cut the DNA at specific places. The fragments are then separated by gel electrophoresis and

blotted onto a membrane. Then a radioactive membrane is used to bind and target the fragments. This creates a pattern of bands that are very distinctive among individuals. Obviously it is not possible to check everyone's DNA every time a murder is committed, but it does provide investigators with a tool and a huge advantage in solving crimes.

How is DNA fingerprinting done? Is it a complicated procedure? When Alex Jeffreys developed this method, he had no intentions of using it for murder investigations. However since 1985, it has been used in over 2,000 cases. Jefferys knew that everyone's genome is different. Therefore, it is possible to give everyone their own so called "fingerprint" using a sample of their DNA. To begin, minute samples of DNA can be obtained from blood, skin, hair, or semen. This sample of DNA can then be amplified using PCR to make millions of copies of the original DNA. Next, the DNA can be cleaved using restriction enzymes.

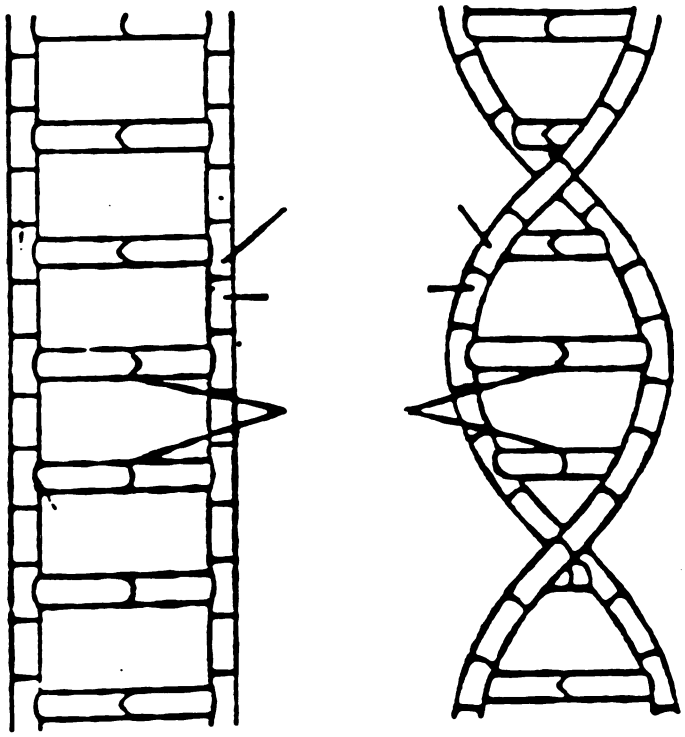
It is important to mention that DNA fingerprinting takes advantage of the fact that large portions of the genome is made up of "junk DNA". Junk DNA is repeated sequences in the human genome that do not code for proteins. Individuals have different numbers and amounts of junk DNA between the DNA that actually codes for proteins. One person may have 4 repeats between two working genes and the next person may have 25 repeats between the same two genes. When these two samples are cut by the restriction enzymes varying lengths of DNA result.

These fragments are then sorted according to their size by the process of gel electrophoresis. Keep in mind that the short fragments travel faster and farther in the gel than the longer ones. The DNA

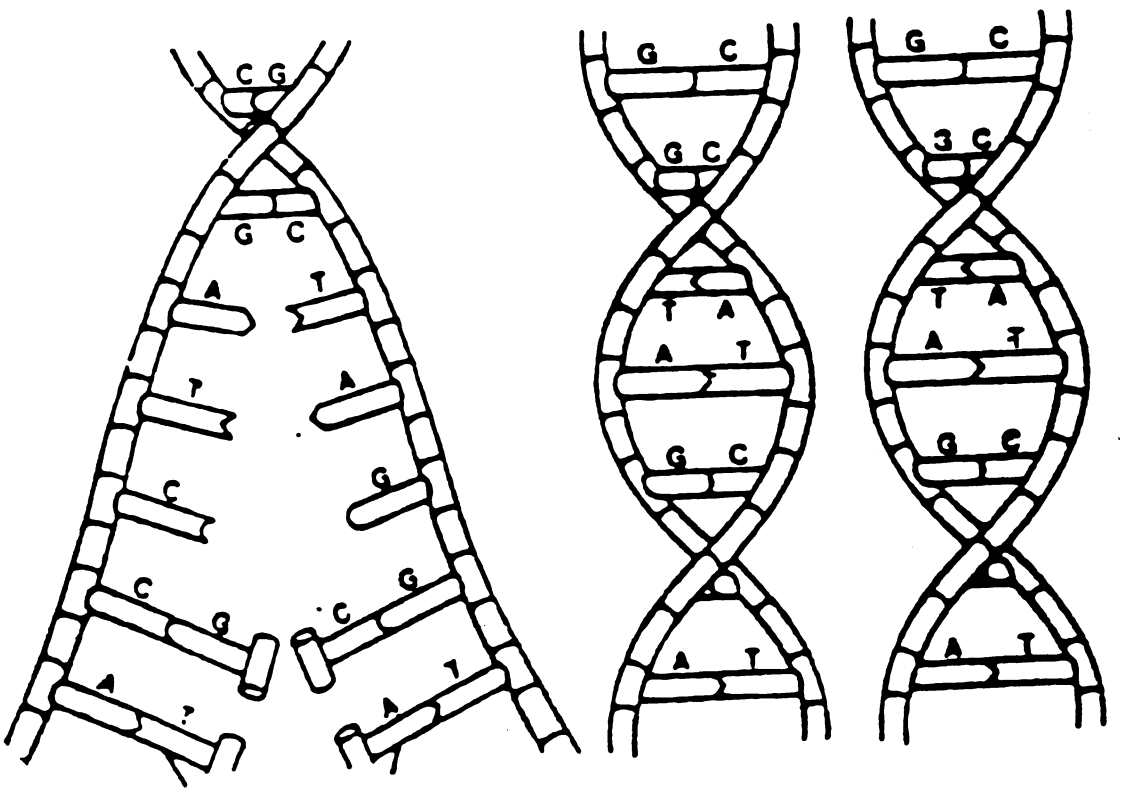
fragments that contain repeats or "minisatellites" are detected by using radioactive probes. The Southern blot analysis is an excellent method for the addition of probes.

These probes are radioactively labeled pieces of DNA whose bases are complementary to those of the repeats. The probes then match with the repeats and stick to them. This method produces a very distinguishing and original pattern of bands - the DNA fingerprint. An example of a DNA fingerprint analysis can be found in the appendix.

Basic DNA Structure Drawing



DNA Replication Coloring



The Isolation of DNA From Onion Tissue
Teacher Section

Introduction: Students are exposed to the concepts of DNA very early on. With the media covering criminal trials and mentioning DNA forensics, the public is being asked to understand DNA science as if it were common knowledge. In school, many students finish their science education without any exposure to biotechnology and recombinant DNA strategies. This lab is merely an introduction to demonstrate the simple methods in isolating DNA from an onion. The students will actually prepare and handle DNA and learn the simple concepts of solubility and precipitate formation.

Background: Many genetic experiments and tests require the removal of DNA from the cell. This procedure can be very simple or quite complex. It depends entirely on the type of cell from which the DNA is extracted. The process reported here is relatively simple as we are isolating the DNA from an onion cell. The teacher should be able to prepare for this lab in approximately 15 minutes as it is broken into 3 separate days. On day one the students will need the last 15 minutes of the hour. Day 2 will need the entire hour. On day 3 the last 20 minutes will be plenty of time to precipitate.

Materials:

Chemicals (Homogenization Medium)	1 liter
Liquid dish soap	30 ml
NaCl	8.8 g
NaCitrate	4.4 g
EDTA	0.3 g
Distilled Water	1 liter

The soap acts to lyse the cells; the NaCl and the NaCitrate provide sufficient ionic strength to force the DNA molecule into an insoluble conformation in ethanol; the EDTA is needed to help inactivate the enzymes that are always present and can be introduced by the students hands. The ethanol is used to precipitate the DNA.

Lab apparatus

Cold ethanol (90 - 100%)
Latex gloves
Blender
60° C water bath
Medium size onion
Cutting board and knife
2 - 250 ml beaker
Ruler
Ice
Cheese cloth
2 - 20 ml test tubes
Wooden applicator
Marking pens

For best results:

* The ethanol must be kept very cold (0 to 10 degrees C). If possible the night before, loosen the cap and place the bottle in the freezer overnight.

* Use a wooden applicator to spool the DNA as it seems to work better than a glass rod.

* To make a simple ice bath, a cake pan with water can be suspended over a bunsen burner. This gives the students something to do for the 15 minute period of heat treating as they can move the burner back and forth under the pan. If possible, have ice on hand to help reduce the temperature of the water bath if it gets too high.

Name_____
Hour_____

THE ISOLATION OF DNA FROM ONION TISSUE

Introduction:

In this lab you will extract DNA from an onion. After the lab you will be able to see and appreciate a procedure for extracting DNA from a living organism and then actually hold the extract in your hand. The concept of solubility and precipitate formation must first be understood. DNA is completely insoluble(not able to dissolve) in 90% ethanol as all of the other parts of the cell will completely dissolve. Therefore when the cell contents are solution (or completely dissolved) and then ethanol is added, the DNA will precipitate or "appear".

It is important that you recall the role of enzymes again. DNA-altering enzymes have the ability to cut the long strands of DNA to the point that you will not be able to appreciate the length of the molecule. So it is especially important that you do not introduce them at any point of the experiment. These enzymes are always present on our hands so it is essential that you practice a technique of wearing rubber gloves throughout the lab even when cleaning the glassware and cutting the onions.

This lab will take 3 days to complete. Each day you will outline the materials needed and the procedures that must be followed in order to isolate the DNA from the onion.

DAY 1

Materials:

Medium sized onion
Cutting board and knife
250 ml beaker
Rubber gloves

Procedures:

To prepare the onion for DNA isolation, put on your rubber gloves and dice a 12 g piece of onion into cubes that are no larger than 3 mm on each side. Place this onion into a 250 ml beaker, cover and store it in a refrigerator overnight.

DAY 2

Materials:

Chopped onion in the 250 ml beaker
250 ml beaker
Homogenizing medium
60 C water bath
Ice bath
Blender

Procedures:

*Transfer the 12 g of diced onion to the clean, room temperature 250 ml beaker and add 50 ml of the homogenizing medium to the onion. Incubate this mixture at 60 C for 15 minutes (NO LONGER). This heat treatment softens the onion tissue and allows the homogenizing medium to penetrate the cell and remove the unwanted enzymes.
*Quickly cool the beaker in an ice bath (a slush of ice and water) for 6 minutes to prevent the DNA from breaking down.
*Next place the contents in a blender at high speed for 75 seconds to lyse (break open) the cell and release its contents.
*Cover and store the beaker in a refrigerator overnight.

DAY 3

Materials:

250 ml beaker containing preparation
Cheese cloth
90 -100% ethanol
2 - 25 ml test tubes
Wooden applicator and marking pens
250 ml beaker

Procedures:

*Filter the preparation through 4 thicknesses of cheese cloth into a clean 250 ml beaker leaving the foam behind. This removes the cell walls and other large unwanted cell parts.
*Cool this filtered solution in an ice bath for 10 minutes.
*While the solution is cooling , label 2 test tubes homogenate (H) and the other ethanol (E). For the test tube that will contain the homogenate, make a line 4 cm from the bottom. The tube containing the ethanol will get a line 5 cm from the bottom.
*Obtain the cold ethanol and pour it in the test tube up to its respective line. Do the same for the homogenization medium.

*Now slowly add the ethanol to the homogenate test tube. A visible precipitate should appear. Using a wooden applicator, spin it in one direction to spool out the precipitate (the DNA).

Questions:

1. If you did not wear rubber gloves during the lab, what could you possibly introduce to the DNA? What would happen to the DNA?

2. What is the significance of adding cold ethanol to the homogenate?

3. Why is it important to rotate the rod in the same direction?

4. What could possibly happen if the onion tissue is heated too long?

5. In what ways could scientists possibly use this process? For example in the fields of forensics or maybe law enforcement.

HOW BIG IS THE HUMAN GENOME
TEACHER SECTION

Introduction:

In this activity, students will understand the enormous amount of information that lies in the nucleus of each human cell. Scientists have calculated the approximate number of base pairs in each nucleus. This astounding number is sometimes a hard concept to grasp. However, if it is related to something that students are familiar with, it helps to visualize this idea.

Imagine trying to type out the base pair sequences in one human cell. How long would it take to type 3 billion letters? Performing this lab, the student can calculate how many pages in a book it would take to type out this information. To make this even easier to understand, the student will use their own biology book to calculate this number.

To further this concept, have the students not only realize the number of base pairs but imagine how small they must be in order to fit in a cell. Further more, ask the students to imagine how many mistakes they would make if they had to type out this code. Be sure to remind them that the cell has to do this every time it divides without making a mistake.

Teaching Tips:

1. At first have the students guess how many pages from their book it will take to type out the code. When they are finished, they will find that it takes about 500 to 800 BOOKS!!
2. Have students choose a page in their book that is primarily writing.
3. This is a good exercise for using their math skills involving scientific notation.
4. Instead of walking the students through the activity, allow them some time to calculate the answers on their own. If they have some problems then offer them some assistance.

Name_____
Hour_____

HOW BIG IS THE HUMAN GENOME

All organisms require a substantial amount of DNA to carry out the instructions necessary to live and reproduce. Because DNA is sub-microscopic it is hard for us to imagine the size of the human genome. This exercise will help us understand its actual size by comparing it with something that we are familiar with, our Biology textbook. The human genome is said to have about 3 billion base pairs. If we were to type these base pairs on a page, how many pages would it take to type the entire genome? Let's find out.

Follow these simple steps and we will soon see how many pages it will take to type the entire genome.

1. Select a page in the book that is mostly printed (no pictures). Page #__

2. Choose 5 lines and carefully count the number of characters. Be sure to count the spaces between the words. Now calculate the average number of characters per line and call this **C**.

C = _____

3. Now count the number of lines on the page which we will call **L**. Multiply the average number of characters on a line times the number of lines on the page.

C x L = (G)

_____ **x** _____ **=** _____

4. Now divide the total genome of a human cell **(3 x 10^9)** by the number of characters on a full page in your biology textbook **(G).** This new number will be called **P**

(3 x 10^9) / G = (P)

_____ / _____ = _____

5. At this time divide the number of pages needed for a human genome by the number of pages in your textbook **(T)**. This will tell you the number of books **(N)** that you will need to record the information in the genome of one human cell.

(P) divided by (T) = (N) !

_____ / _____ = _____

N represents the **total number of books** it would take to write out the entire human genome from just one cell.

WOW, the human genome is HUGE ..... or is it very, very small?

HOW GENES MAKE PROTEINS
"Transcription and Translation Simulation"
Teacher Section

Background Information:

In order for traits to be expressed, protein synthesis must take place. Students need to realize for DNA to have instructions carried out, it has to be transcribed into a substance (RNA) that has the ability to leave the nucleus and ultimately translate its message to the cell. In this way, proteins can help to carry out almost all of the processes of life. Remember, DNA is carrying a message, that is a genetic code. That genetic code is information to instruct the proteins to determine the structure and function of organisms.

Protein synthesis takes several steps and can get quite confusing, so it helps to use simulations to walk the students through the process. In this activity the students will act out the steps of transcription and translation to help clarify the sometimes confusing concepts of DNA science. It actually helps them make sense of what processes occur inside and outside of the nucleus, on the ribosome and what biochemicals are involved.

Materials:

Photocopy four masters of the DNA, mRNA, tRNA and the amino acids using colored papers. Try to use four different colors. The students can cut them out. They are found in the figures section, figures 6 through 12 (pages 88 - 95).

Teaching Tips:

1. Have the students read the introduction and then discuss it with them. Then continue to have them read the rest of the lab before starting.

2. It works best if the instructor helps them walk through the lab to alleviate confusion.

Name_____
Hour_____

HOW GENES MAKE PROTEINS

"Transcription and Translation Simulation"

Objective:

Using the entire class, we will act out the entire process of protein synthesis including transcription and translation.

Materials:

scissors
master cards

Introduction:

This simulation will help give you a better understanding of how the order of the bases in a strand of DNA are translated into a sequence of amino acids to form proteins. When you hear the statement that "DNA is ultimately involved in expressing your traits", you have to wonder what is directly responsible. The answer is proteins. Proteins provide the structural and functional basis of life. They have a role in every possible life function.

There are structural proteins that help make up cartilage and tendons and others that make up our hair and eyes. Enzymes that catalyze chemical reactions and hormones that regulate biochemical messages are all examples of proteins. Other proteins such as hemoglobin, antibodies, nutrient storers and waste transporters all have important functions.

The process of making these proteins, known as protein synthesis, is complicated but after you follow the steps in this activity it will become clear that DNA is a template, used to mold or pattern the order of the amino acids. Keep in mind, single stranded RNA makes a copy of the desired genes and migrates from the nucleus to the ribosome where it can dictate the sequence of the amino acids.

Procedure:

A. Setting the Scene:

1. The classroom is going to be our cell. Imagine the walls, floor and the ceiling as the cell membrane.
2. The area of the room that has the chairs in a circle represents the nucleus of the cell. This is where transcription occurs. Remember, mRNA will have to move in and out of this area.
3. The rest of the room is the _____ .
4. The other area of the room designated ribosome is where translation occurs.

B. Distribution of Materials:

1. The teacher will now distribute DNA sequences and their complementary mRNA strands to two groups of students. The cards each have three letters. Remember that the bases must be in groups of three as they code for triplet base units. The letters on the cards refer to the bases. A is adenine and so on.

C. Transcription:

1. Now assume the DNA strand begins to unzip. The double strand actually becomes two strands. Only one of the two strands will be used. We call this the active strand, while the other strand is the dummy strand.
2. All of the students with the DNA cards will now line up inside of the nucleus. The "TAC" card will line up at the left. This is the start code. And the "ATC", the stop sequence will be in the right. The rest of the cards may line up in any order.
3. Next, RNA polymerase helps to pair the exposed part of the DNA's bases with the complementary RNA bases. At this point, students with the mRNA cards should go into the nucleus and pair with its complementary DNA base triplet. This three base mRNA molecule is called a codon. Remember: **RNA guanine pairs with DNA cytosine
   **RNA uracil pairs with DNA adenine
   **RNA cytosine pairs with DNA guanine
   **RNA adenine pairs with DNA thymine

4. When the mRNA strand has been made and placed in correct order, the students holding the DNA cards may be seated. You have just acted out the process of transcription. Notice what was just made, a strand of mRNA that reflects the exact opposite of the DNA code.

D. Translation:

1. Notice that the tRNA cards are arranged in groups of three letters. These three base sequences of tRNA are called anticodons. Also these tRNA cards have a three letter abbreviation contained in the arrow. This represents one of the 20 amino acids. Now, distribute the tRNA cards and the amino acid cards to the rest of the students and randomly scatter the students throughout room.
2. Have the students in the nucleus exit while staying in the same order. Move to the part of the room designated ribosome. At the same time, the students with the tRNA cards need to find their corresponding amino acid. For example the tRNA anticodon card "GCG" with the letters PRO should find the amino acid Proline.
3. After the tRNA cards find their corresponding amino acid, both students should migrate towards the area of translation, the ribosome, and look for the mRNA card that matches their tRNA card. Use the rules of complementary base pairing:
   - **RNA adenine always pairs with RNA uracil
   - **RNA uracil always pairs with RNA adenine
   - **RNA cytosine always pairs with RNA guanine
   - **RNA guanine always pairs with RNA cytosine
4. Use the following table to check for the correct pairs. Except for the start and stop cards, the following sequences will probably not be in the same order.

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

5. As each tRNA anticodon finds its corresponding codon on the mRNA strand, the tRNAs detatch from their amino acids. All of the amino acids should remain intact, especially their order. At this point in time the amino acids will form a peptide bond between each one, thus forming a polypeptide. Translation is now complete. Remember, translation is when a sequence of mRNA information translates into a polypeptide.

E. Questions:

1. List three different proteins and their functions

   a.

   b.

   c.

2. Why do we say DNA is indirectly involved in determining structural arrangement of proteins.

3. Suppose an individual has a nutrient deficiency due to a poor diet and is missing a particular amino acid. Which process of protein synthesis would be more affected and why?

4a. Compare the active and the dummy strands of DNA.

b. Would there be a problem if the dummy strand was used to make proteins

5. Below are some tRNA anticodons/amino acid relationships and a strand of imaginary DNA. Please fill in the missing boxes in the chart below by writing the correct mRNA codons, tRNA anticodons and amino acids.

Use the following tRNA/amino acid relationships:

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

DNA	TAC	GAT	AGG	GAT	CAG	TTA	CTC	GGC	ATC
mRNA									
tRNA									
Amino Acid	met start								stop

5. A new and exiting branch of biotechnology is protein engineering. To make or engineer proteins, molecular biologists work backward through a protein synthesis process. They first determine the exact sequence of the polypeptides they want and then create a DNA sequence to produce. Fill in the rows for tRNA anticodons, mRNA codons, and DNA.

Use the following tRNA/amino acid relationships:

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

DNA											
mRNA											
tRNA											
Amino Acid	met start	leu	val	pro	gly	asn	ser	glu	glu	pro	val stop

AGAROSE GEL ELECTROPHORESIS USING DYES
TEACHER SECTION

Introduction: This lab exercise is an excellent way to introduce the techniques of gel electrophoresis. It demonstrates the simple ideas of how molecules can be separated according to their size. Most of the equipment has been generated by other teachers and graduate classes through Michigan State University. Instead of using DNA fragments, the students will be using a series of easily available dyes and running them through a gel towards an electrode in an electric field. The results will show that the dyes are various sizes and will travel towards the anode (+) or the cathode (-). An unknown dye mixture will also add a section to the lab that will allow the students to make comparisons and differences between the dyes. As you will see, the unknown mixture dye will separate into three single dyes as the students can then try and estimate the molecular weights of the dyes by comparing them to the known dyes.

Background: With the ongoing task of developing lab exercises that challenge and appeal to students, it is difficult at times to keep the cost down. This lab was developed with this in mind. Most of the equipment that is used is inexpensive. The power sources are probably the most expensive item and usually the teacher can borrow these from the physics department or their local college for a nominal fee. As for the chemicals, they can all be purchased through any scientific company.

The ability to learn about biotechnology is paramount in the classroom. Learning about the chemistry of DNA is not enough. The students should be exposed to concepts and techniques that are current. They hear about DNA fingerprinting on the news quite often. It is a shame that they have to learn about these issues on TV instead of the classroom. It is up to the teacher to make the commitment to keep their teaching strategies up to date and not fall into a rut and omit important labs because they are too expensive. Most labs can be recreated by using simulations as this lab proves.

Although DNA is not used in the lab exercise, it shows the basic principles of how DNA fragments can be separated according to size. The gel chambers are modified Rubbermaid containers. The buffer can be made in just minutes and the gels can be loaded by a beginner. Results can be seen in about 30 minutes.

Materials:

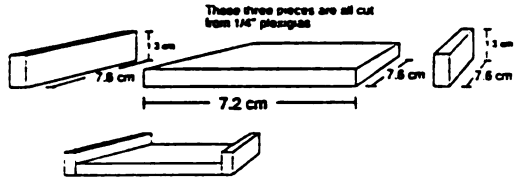
1. The electrophoresis chambers can be made by purchasing a 10 inch x 5 inch Rubbermaid container. Cut a square in the top of the lid about 6 cm square. Use carbon rods 1 cm in diameter and about 3 cm long. Using a cork borer, poke 2 holes in the top of the lid on each end and place the carbon rods in the holes. Next drill a hole in the ends of the carbon rods that stick above the top of the container just big enough for a nail to slide in about half way.

2. The 50 amp power supply must be a DC power supply. Up to four gels can usually be run on them.

3. Tris-Borate Buffer (TBE) can be made by adding 10.8 g of Tris base and 5.5 g of boric acid and 20 ml of 0.5 M EDTA to 1 liter of distilled water.

4. A total of 2 gels can be made by adding 0.5 g of agarose to 70 ml of buffer in an Erlenmeyer flask. Bring the flask to boil then set aside for two minutes to cool.

5. The gel trays can be made using three pieces of plexiglass and gluing them together to look like the picture below.



6. The dye samples can be made by simply dissolving 0.5 g of dye in 100 ml of distilled water. Next add 1 ml of glycerine to make the solution dense enough to sink in the buffer when loading the gels.

For best results:

* A good stopping point is between steps 1 and 2. After pouring the gels simply cover with saran wrap and place in the refrigerator overnight.

* The recommended mixture for the unknown dyes is to combine orange-G, bromophenol blue, pyronin-Y at a 1:1:1 ratio

Name_____
Hour_____

AGAROSE GEL ELECTROPHORESIS USING DYES

OBJECTIVE:

To understand and use one of the most utilized techniques in separating DNA fragments: Agarose Gel Electrophoresis.

BACKGROUND:

The agarose gel is a semi-solid solution that resembles "Jello" and is allowed to solidify in the gel tray. Briefly, the students will be loading 7 different dye samples into small holes made in the gel. These holes will be referred to as "wells". After the dyes are loaded into the wells a power supply will be connected to the opposite ends of the gel. The dyes will immediately begin migrating ***through*** the gel towards a specific electrode. It is important that students understand the principles involved with gel electrophoresis. To put simply, the larger the molecule traveling through the gel, the slower it will travel. This is exactly how DNA fingerprinting is done. If a gene fragment is smaller than another fragment, it will run faster and farther through the gel. In this manner one DNA fragment can be separated and compared to another fragment.

Using the dyes of different molecular weights in place of the gene fragments is an excellent way to show the same principles. The movement of the dyes in an electric field can be used to show that:

(1) the migration direction of the dyes can be used to show its overall negative ***or*** positive charge. For example, if the dye migrates towards the positive electrode (called the anode) the overall net charge of the dye molecule is negative.
(2) Some dye's molecular weight is more than others by moving slower through the gel.

(3) Some dyes can be used as standards to calibrate an agarose gel. Dyes such as xylene cyanol, bromophenol blue, and orange-G are accurate size markers for small DNA fragments. Remember DNA fragments have an x-amount of base pairs. The "Base Pair Equivalents" for these marker dyes are as follows:

DYES	BASE PAIR EQUIVALENTS
Orange-G	70
Bromophenol Blue	250
Xylene Cyanol	2800

Materials: Each team of three students will need:

-Electrophoresis chamber
-@50 amp power supply
-Gel tray
-Gel comb
-600-ml beaker
-100-ml graduated cylinder
-325 ml Tris-Borate-EDTA Buffer (TBE)
-300-ml Erlenmeyer Flask
-0.5 g Agarose ***(makes 2 gels)***
-Heat Source (Bunsen Burner works fine)
-Micropipette and rubber bulb
-Dye Samples
#1 orange-G
#2 pyronin-Y
#3 phenol red
#4 bromophenol blue
#5 crystal violet
#6 xylene cyanol
#7 unknown dye mixture

Procedure:

1. Agarose preparation: *Makes 2 gels*

a. Tape the ends of the gel tray as described by the teacher.
b. Next tape the comb into place as described by the teacher. Be sure to place the comb in the middle.
c. Place 0.5 g agarose in the 300-ml Erlenmeyer flask and then add 70 ml of TBE buffer.
d. Place the flask on the heat source and bring the solution to a mild boil until all of the agarose has dissolved.
e. Allow the solution to cool for about 3-5 minutes.
f. Using a pasteur pipette, lay a thin bead of agarose where the tape meets the tray and allow to cool and solidify.
g. Next pour the agarose into the tray to about 1-2 mm from the top and allow to cool until the gel has solidified.

2. Loading the Electrophoresis Unit:

a. Carefully remove the comb from the gel. This will leave small holes in the gel. These small holes are called "wells".
b. Remove the rest of the tape on the ends of the tray.
c. Place the gel tray in the electrophoresis box.
d. Fill the box just to the very top edge of the gel with TBE buffer.
e. Very carefully add the dyes using the micropipette to the wells. Approximately 5 microliters of each will be sufficient. **Be sure to draw and label a diagram of the order of the dyes**. The order from the materials list works great.

NOTE: The dyes are more dense than the buffer, so they will sink to the bottom of the wells. ***Be sure NOT to overflow the wells***.

3. Running the Electrophoresis:

a. Carefully place the top on the box
b. Connect the power supply to the electrophoresis top. Be sure not to turn on the power until all leads are connected. Attach the positive electrode (red) to the side of the tray that has 2/3 of the gel exposed. The negative lead should be attached to the side of the tray that has only 1/3 of the gel. Turn on the power supply and adjust it to about 30 volts.
c. Closely watch for the initial migration of the dyes to be sure all of the connections are satisfactory. The colored dyes should move out of the well and into the agarose gel.
d. Immediate observations can be made about the net overall charge of the dyes but the power should be left on until the dye mixture (#7) separates into 3 distinct colors.

Data and Analysis: Record your observations in the chart below.

DYE	LENGTH OF MOVEMENT(mm)	NET OVERALL CHARGE
#1 orange-G		
#2 bromophenol blue		
#3 pyronin-Y		
#4 crystal violet		
#5 phenol red		
#6 xylene cyanol		
#7 dye mixture		

DISCUSSION AND QUESTIONS:

1. What dyes are positively charged and which electrode did they migrate towards?

2. Compare the size of gene fragments in terms of the speed that they might travel through the agarose gel.

3. What were the three unknown dyes in the dye mixture?

4. Of the three dyes that were used as standards, which one of them is probably the biggest molecule and how can you tell by looking at your gel?

5. Approximately how many base-pair equivalents would you guess phenol red to be? (Hint: compare it with your standards).

PALINDROMES
BACKWARDS OR FORWARDS
Teacher Section

Introduction:

A group of words or letters that read the same forwards and backwards are called palindromes. These palindromes can occur in DNA sequences. This is precisely where most restriction enzymes make their cuts: within the palindrome. These chemical scissors travel along the DNA strand from the 5' end to the 3' end until they recognize a specific restriction site. These restriction sites involve two specific bases within a very specific palindrome. Each individual restriction enzyme cuts at different palindromes.

For example, the restriction enzyme EcoRI cuts at the palindrome G A T C, between the letters A and G. Since the DNA is double stranded, you will notice that a staggered cut is made. Remember that DNA is antiparallel so the enzyme reads the top strand from left to right and right to left on the bottom strand. This staggered cut actually produces a place for the host DNA to attach. Thus these attachment sites are called "sticky ends". The name refers to the way the cut DNA readily attaches itself to the vector.

Teacher Tips

1. Each student will need a copy of the lab.

2. Other restriction enzymes that can be used:

**Bam HI - uses the site between G and G in the palindrome

5' G~GATCC 3'

**HIND III - uses the site between A and A in the palindrome

5' A~AGCTT 3'

**Hpa II - uses the site between C and C in the palindrome

5' C~CGG 3'

Name_____
Hour_____

PALINDROMES

BACKWARDS OR FORWARDS??

Introduction:

Get ready! Genetic engineering is here: DNA technology at its best. There is a lot of excitement in this growing field and it is having an impact on all of us. What is this branch of biotechnology? To put it simply, DNA sequences or genes are being removed from one organism and placed into another.

But why would we want to do this? It's easy. To lower the costs and increase the availability of a protein based product. For example, everyone knows a diabetic person. Where do these diabetics obtain their insulin? From products made by genetic engineering. To begin, we must realize that there is a human gene responsible for instructing the cell to make insulin. If we can remove that gene from a human cell and place it in a host organism, it will make insulin for those individuals that can't.

For example, scientists have isolated the gene that codes for insulin production. They can remove it from a human cell using restriction enzymes acting like scissors. This enzyme removes the gene from the cell to be placed in its new host cell. In this case, the host cell happens to be a bacterial cell. Why a bacteria cell? Because bacteria are easy to obtain and they reproduce very rapidly. The bacteria cell can divide, making many new cells with the insulin gene in it. Ultimately the bacteria starts making insulin as instructed by its new gene.

But how does the restriction enzyme know exactly where to find this gene and then cut it out? To begin, we have to know what restriction enzymes are. Normally, bacteria use these enzymes to prevent or stop the invasion of foreign DNA into their cells. Basically these enzymes chop up this DNA so it is ineffective in their cells. Scientists learned that these enzymes cut up DNA in very specific places.

The sites where these enzymes cut the DNA are called restriction sites. But how do the enzymes recognize these sites? The answer is the notion of **Palindromes**. A palindrome is a phrase, word or sequence of letters that reads the same **forwards and backwards**, like the words radar, pop, or madam. Restriction enzymes only cut within a palindrome in one direction. The direction is usually from the 5' end to the 3' end.

Use the palindrome: a man, a plan, a canal, Panama. Imagine that the enzyme only cuts between the A and M reading from left to right. How many pieces would your sentence be if you allowed the enzyme to do its work? Write it out in the space below and find out. Palindromes can also be found in DNA. Since DNA is double stranded, this method works perfectly. Look at the palindrome below

5' G G A T C C 3'

3' C C T A G G 5'

The top strand reading left to right is the same as the bottom strand reading right to left. One specific restriction enzyme cuts at this palindrome between the G and G. This is what the cuts would look like:

5' A A T C G C G~G A T C C G C T T A A 3'

3' T T A G C G G C T A G~G C G A A T T 5'

The restriction enzyme caused a staggered cut. This staggered cut is referred to as "sticky ends". These sticky ends are what allow genetic engineers to successfully cut and splice genes. All that is needed is to make this same cut in the host cell and these sticky ends will fit right into place.

Scientists have discovered many restriction enzymes. The above enzyme is called BamHI, referring to the bacteria that it was isolated from.

Examples

Be sure to read all the steps before starting. Use a separate sheet of paper for the answers.

1. The palindrome for EcoRI is GAATTC. It cuts between G and A inside of the palindrome. Write out the palindrome (recognition site) as a double strand of DNA.

2. Make up a sequence of 15 bases using T-A-G-C in any order. Within this sequence, add the recognition site of EcoRI in two different places.

3. Now cut the DNA sequences by using an arrow. Be sure you have "sticky ends".

Questions

1. Why are palindromes important to genetic engineers?

2. Where do restriction enzymes come from?

3. Compare and contrast DNA and genes.

4. Write a description of a "sticky end" and explain why it is important to have this type of end when doing genetic engineering.

5. What kinds of solutions to problems in disease fighting do you think can be solved through genetic engineering?

6. What kinds of problems do you think genetic engineering might cause in the future?

CONSTRUCTING A RESTRICTION ENZYME MAP

Teacher Section

Introduction: Recombinant DNA technology is a growing field of science, growing so fast that many teachers can't keep up with the new techniques. Recombinant DNA is DNA that is made by connecting fragments of DNA from different sources. Restriction enzymes are used to cut DNA at specific locations which then can be removed from the cell. Once any kind of recombinant DNA has been cut and removed, it can be inserted into another organism as that organism can now use the foreign DNA as if it were its own. Insulin production from bacterial cells is a good example.

Background: This lab activity will demonstrate two main principles: the first being that restriction enzymes cut or "cleave" DNA at very specific recognition sites and the next being that these resulting strands of DNA are different lengths. Therefore they travel at different speeds in agarose gel during electrophoresis.
Students will find out that there are many restriction enzymes that may make one or more cuts in a strand of DNA. The cuts are given on a chart based on how many base pairs that particular strand possesses. The main idea is that the more the base pairs in a DNA segment the slower it travels through a gel.

For best results:

* The pop-it-beads can be purchased beforehand from almost any science catalog.

* This lab activity takes about a 50 minute period.

Name_____
Hour_____
Date_____

CONSTRUCTING A RESTRICTION ENZYME MAP

Objective: Students will construct a restriction enzyme map using materials provided. Using the data, the students will know where restriction enzymes have cut or "cleaved" plasmid DNA.

Materials: paper
pencil
pop-it-beads
17 cm diameter cylinder (poster board works great)
colored pencils
calculator

Procedures:

Part A

1. Table 1 represents the molecular weight standards for a plasmid. This plasmid has a total of 1000 base pairs. Using gel electrophoresis, it has been determined that a segment of DNA of 900 base pairs travels 0.80cm on the gel.

a. Since a molecule with 400 base pairs is smaller than one with 900 base pairs, it can travel farther and faster on the gel. How far did 500 base pairs travel? _____

b. How far did 100 base pairs travel? _____

2. Table 2 shows what happens when each restriction enzyme is placed alone with the plasmid and allowed to cut it at it's restriction site.

a. How many cuts did enzyme A make?_____

Enzyme B_____, Enzyme C_____

3. Using yellow pop-it-beads, construct a model of the plasmid . Each bead will represent 20 base pairs. Ignore the cuts in the plasmid.

a. How many total base pairs does the plasmid have?_____

b. If each bead represents 20 base pairs, how many beads do you need for this plasmid?_____

c. At this point, fill in tables 2 and 3.

4. Starting with enzyme B, notice that it only makes one cut. This fragment represents the total length of the plasmid. Now using your plasmid that you constructed in procedure 3, remove 2 yellow beads and insert a pair of blue beads to represent the cut made by restriction enzyme B. This is plasmid #2. Does it matter where you put the blue beads?_____

5. Construct a second plasmid using green beads to represent the cleavage site for enzyme C. This is plasmid #3.

6. Construct a third plasmid (actually plasmid #1), this time using red beads to represent the cuts made by enzyme A. Remember this enzyme makes two cuts. The red beads should be placed so that they are separating the plasmid into 2 separate sections.

a. How long is the first segment?_____

b. How long is the second segment?_____

7. Table 3 shows the results of mixing two of the restriction enzymes together and allowing them to cut the plasmid.

a. How many cuts did enzymes A and B make?_____

A and C_____, B and C _____

You may now take apart the 3 plasmids you just made. Save the beads for the next section.

8. The next 3 plasmids that you will construct will have several cuts caused by two enzymes. Build plasmid 4 that has been cleaved by enzymes A and B from table 3 after answering these questions.

a. How many cuts did enzyme A and B make?_____

b. Which of these cuts are still the same from enzyme A?_____

c. Which enzyme A base pair was cut?_____

d. When making plasmid 4 how many base pairs long will each section be?_____, _____, _____

9. Now construct plasmid 5 where A and C made 3 cuts.

a. Which of those cuts is still the same from enzyme A?_____

b. Which enzyme A base pair was cut?_____

c. When making plasmid 5 how many base pairs long will each section be?_____, _____, _____

10. Now construct plasmid 6, where enzyme B and C made cuts.

a. How many cuts are made?_____

Part B

1. If you have reached this point successfully, GREAT JOB ! Now notice you have 3 plasmids. Do not lose track of which plasmid is which. Remember that plasmid 4,5 and 6 have been cut by 2 enzymes so they should have 2 or 3 restriction (cut) sites. **Now** stack each of these plasmids on the ring. Rotate the three plasmids until all of the beads of the same color (except yellow) are directly above each other. (HINT: You may have to remove a plasmid and flip it over to make a perfect match)

2. "THE SUPER PLASMID" Now construct the final plasmid that shows the restriction sites for all of the enzymes that you used. This plasmid represents a complete restriction map for your plasmid. Using your colored pencils, please transfer this plasmid on to figure 1.

Figure 1

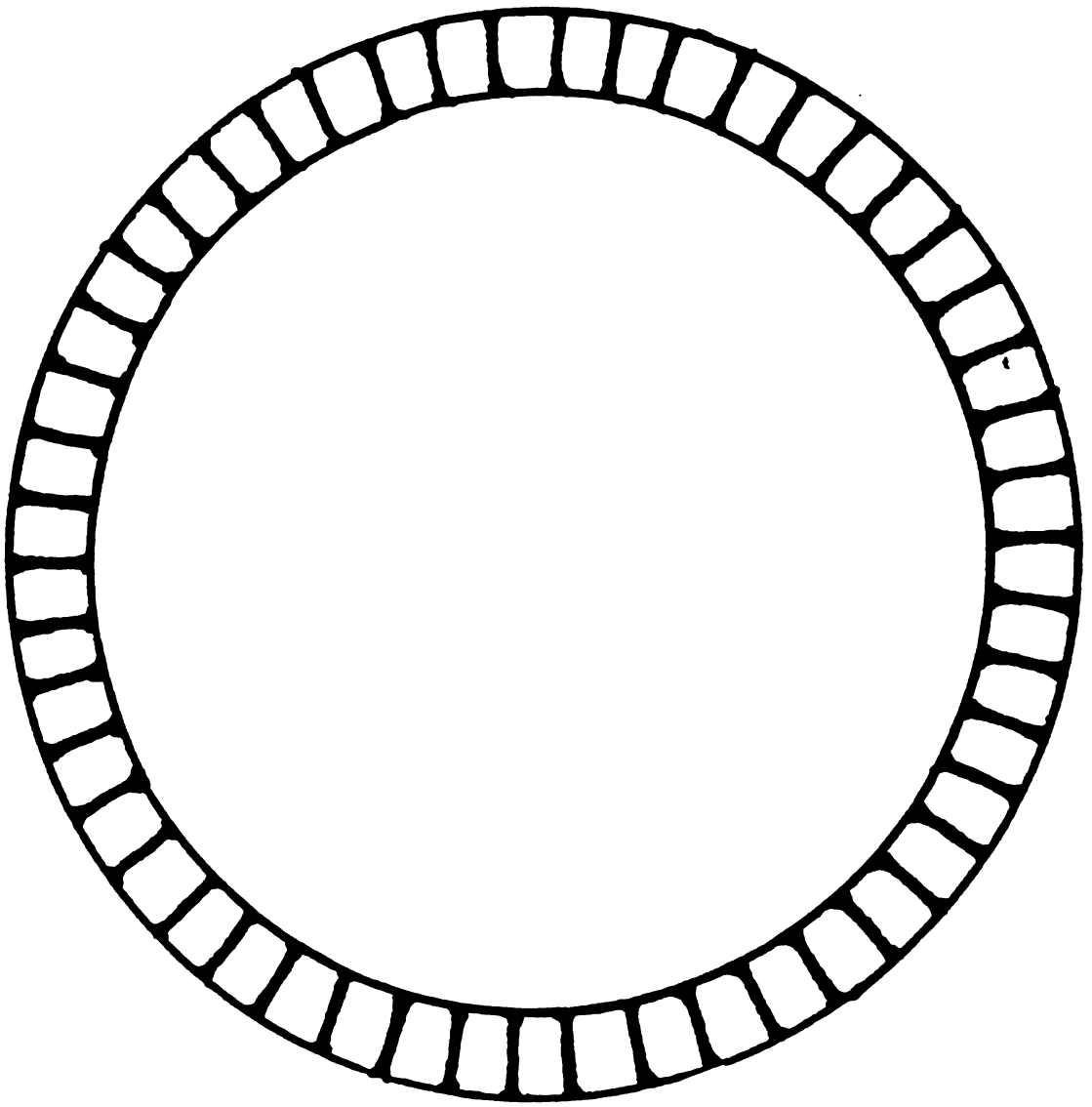


Table 1: Molecular Weight Standards

Base Pairs (bp)	Centimeters
900	0.80
800	1.80
700	1.30
600	1.60
500	1.85
400	2.30
300	2.80
200	3.50
100	4.80
30	7.00
17	8.00

RESTRICTION ENZYME CLEAVAGE POINTS

Table 2: Single Cut Sites

Enzyme A (Plasmid 1)			Enzyme B (Plasmid 2)			Enzyme C (Plasmid 3)		
Beads	bp	cm	Beads	bp	cm	Beads	bp	cm
____	600	1.60	____	1000	0.60	____	1000	0.60
____	400	2.30						

Table 3: Double Cut Sites

Enzyme A & B (Plasmid 4)			Enzyme A & C (Plasmid 5)			Enzyme B & C (Plasmid 6)		
Beads	bp	cm	Beads	bp	cm	Beads	bp	cm
____	280	2.90	____	340	2.70	____	540	1.70
____	120	4.45	____	260	3.05	____	460	2.00
____	600	1.60	____	400	2.30			

MAKING A RECOMBINANT PLASMID
TEACHER SECTION

Introduction:

As teachers, we are constantly being asked to bring technology into the classroom. And for good reason. Students need to be updated on current issues in biotechnology as the field is growing at great speeds. It is not acceptable to know only the structure and function of DNA. Students also need to know about the techniques used by scientists to alter or reconstruct DNA.

In this lab, the students will learn about one such technique. Recombinant DNA techniques are being developed at an astounding rate and the students need to be aware of such basic methods. Recombinant DNA usually involves a specific strand of DNA that is producing a desirable trait or biochemical product and then removed from its host. This process of removing the desired gene is done with a chemical pair of scissors called restriction enzymes. These enzymes can cut the genes from the original strand of DNA with precise accuracy. Next the gene can be placed in a host, usually bacteria. Using the same restriction enzymes, the new DNA becomes incorporated into the host's DNA. As the host reproduces, each new cell has a copy of the introduced gene. At this point the new gene instructs the host to produce the desired product. When the students are finished with this lab, they will discover that:

1. desired DNA sequences can be removed from a cell.
2. the sequences are "cut out" by enzymes.
3. these enzymes are very specific as to where they make their cuts.
4. the desired gene can be placed into a host vector (sometimes called a plasmid).
5. the vector infects a host cell which incorporates new DNA into its own DNA
6. the host cell is now ordered to carry out the instruction of the gene.

Materials: For each group of two students

1 blue sheet (plasmid)
1 green sheet (cell DNA)
1 yellow sheet (restriction enzymes)

Teaching Tips:

1. remind students that the enzymes read from 5' to 3'

2. When making copies of the colored cards, enlarge them just big enough where they can fit on a sheet of paper.

3. Use a large piece of paper when drawing the plasmid.

Name_____
Hour_____

MAKING A RECOMBINANT PLASMID

Introduction:

Making intelligent and responsible choices is something that we are asked to do everyday. Becoming educated about the world around us is paramount in helping us make these decisions. This activity will introduce us to an issue that has made a huge impact on our society. Learning about the pros and cons of biotechnology, can supplement our knowledge about many areas of science.

Recombinant DNA technology, an area of biotechnology, is a growing field of science. This technology uses living organisms to produce substances of interest: combining biology with chemistry and science with industry.

How does recombinant DNA technology work? Recombinant DNA technology involves using enzymes called restriction enzymes to cut out pieces of DNA from one organism. The same enzyme is then used to cut a circular piece of bacteria DNA called a plasmid. The DNA from the first organism is then mixed with the plasmid DNA. The two pieces fit together by another enzyme called a ligase. The resulting plasmid now has the DNA from a different organism. The plasmid is then placed into the bacteria and allowed to reproduce. These resulting new cells are clones of the cell that contains the new DNA.

Today, this is how human insulin is made. The DNA for making human insulin is cut out and inserted into a plasmid. The plasmid is then replaced back into the bacterial cell and allowed to reproduce. Each new daughter cell now has the ability to make insulin. In this way insulin is able to be produced simply, safely and cheaply. This process sounds confusing but it is actually quite easy. This exercise will illustrate the process.

Materials:

scissors
plasmid sheet (blue)
cell DNA sheet (green)
enzyme sheet (yellow)
scotch tape

Procedures:

Part A

1.The strips on the plasmid sheet are written with the DNA 3' to 5' from the top to bottom on the left - hand side of the strip and from the 5' to the 3' from the top to the bottom on the right - hand side. Cut out the plasmid strips along the dotted lines. Do not throw away the key at the bottom of the page.

2. Tape the strips together end to end in any order. Then tape the two ends together to give you a circular plasmid.

Part B

Locate the restriction sites on your plasmid according to the steps below. The enzyme sheet shows how 8 different restriction enzymes will cut base pairs.

1. Cut the enzyme sheet cards

2. Compare the sequences of the base pairs on the cards with what you have on your plasmid. Mark a starting point on your plasmid and work your way around, marking the restriction sites as you find them. Include how it cuts and the name of the restriction enzyme.

3. Using your starting point as a reference, divide the circle in fourths by folding it in half, and then in half again. This will help place your restriction sites, and replication origin in relative distance to each other.

4. Draw a circular map of your plasmid. Marking the restriction sites and replication origin in relative distance to each other. This is called a restriction map.

Part C

1. Keeping the numbers attached, cut out the strips on the cell DNA sheet. This contains the gene you will transfer to the plasmid. Do not throw away the key at the bottom.

2. Assemble the strips in the order one through six as indicated on your handout. You should end up with a long, flat strip. The numbers should not be visible.

3. The gene is in triplet code on the 5' to 3' strand and starts with methionine and ends with a stop codon.

4. Determine which restriction sites are near the gene on your cell DNA. Pick the restriction enzyme that will cut the DNA both above and below the gene.

5. Using the same restriction enzyme, see where it cuts the plasmid. As long as you do not cut the replication origin, you may use the enzyme.

6. Mark your cell DNA to show how the restriction enzyme will cut it.

7. Cut both plasmid and cell DNA following the cutting patterns on the appropriate restriction enzyme cards.

8. Match up the sticky ends of the gene with those of the plasmid and tape the ends together. WAALAA!! RECOMBINANT DNA!!

What would scientists do next?

1. The recombinant DNA would be inserted into a _____and be allowed to divide.

2. The bacterial cells are then screened for the inserted gene.

3. Those that possess the inserted gene are grown and the gene product is collected. For example, insulin.

QUESTIONS:

1. What restriction enzyme did you use?

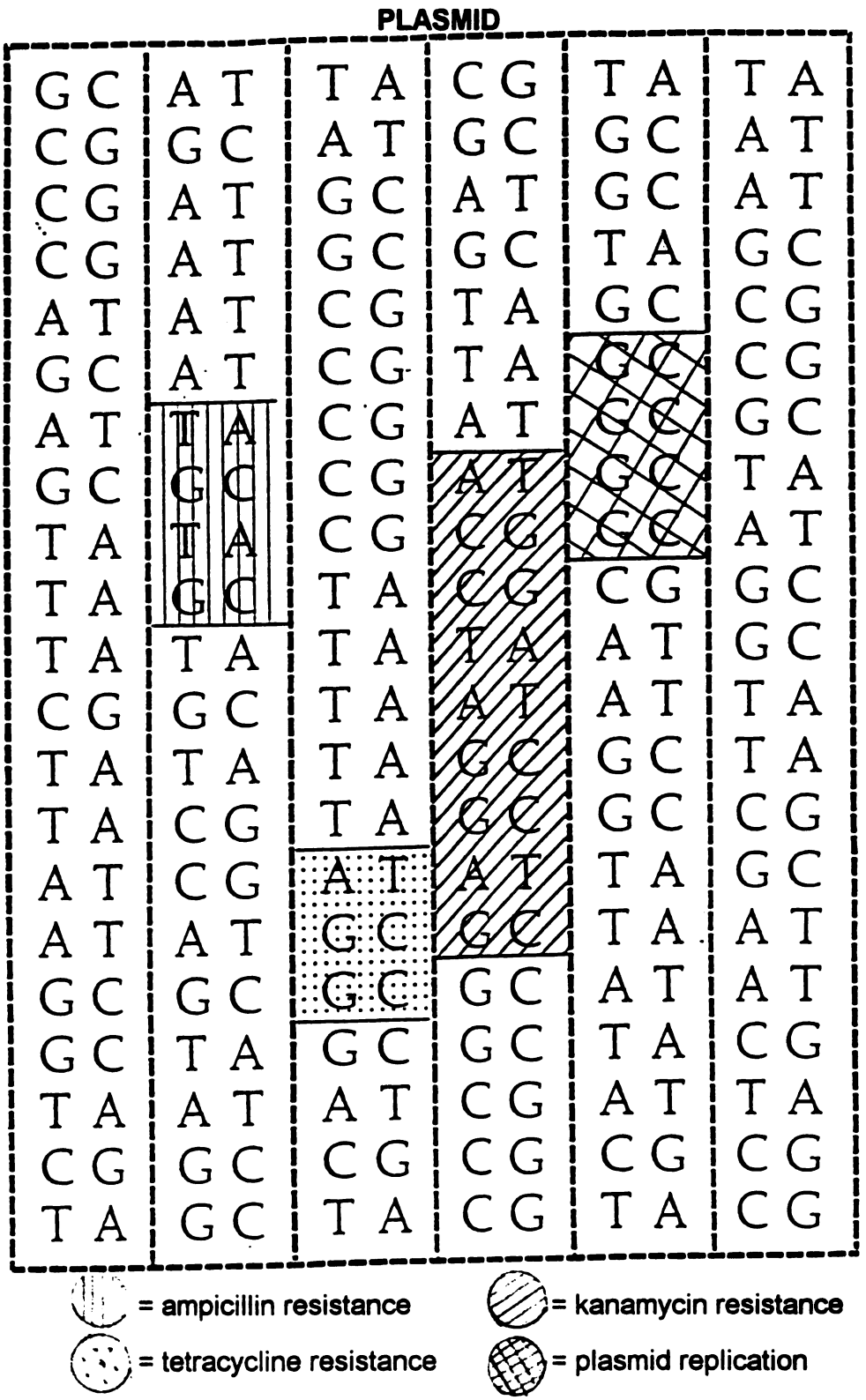
2. What does the ligase card allow you to do?

3. What do restriction enzymes do?

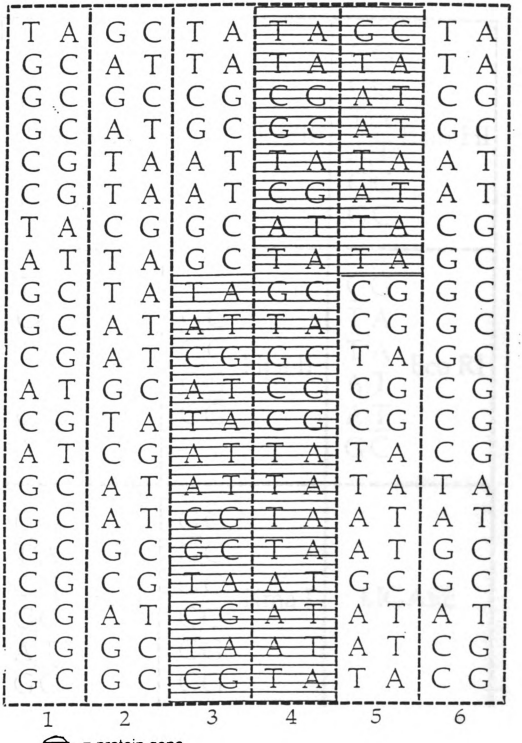
4. Why must you use the same restriction enzyme for both the cell DNA and the plasmid?

5. This new plasmid is called a recombinant plasmid. What does this mean?
(Hint: think of the word "recombinant")

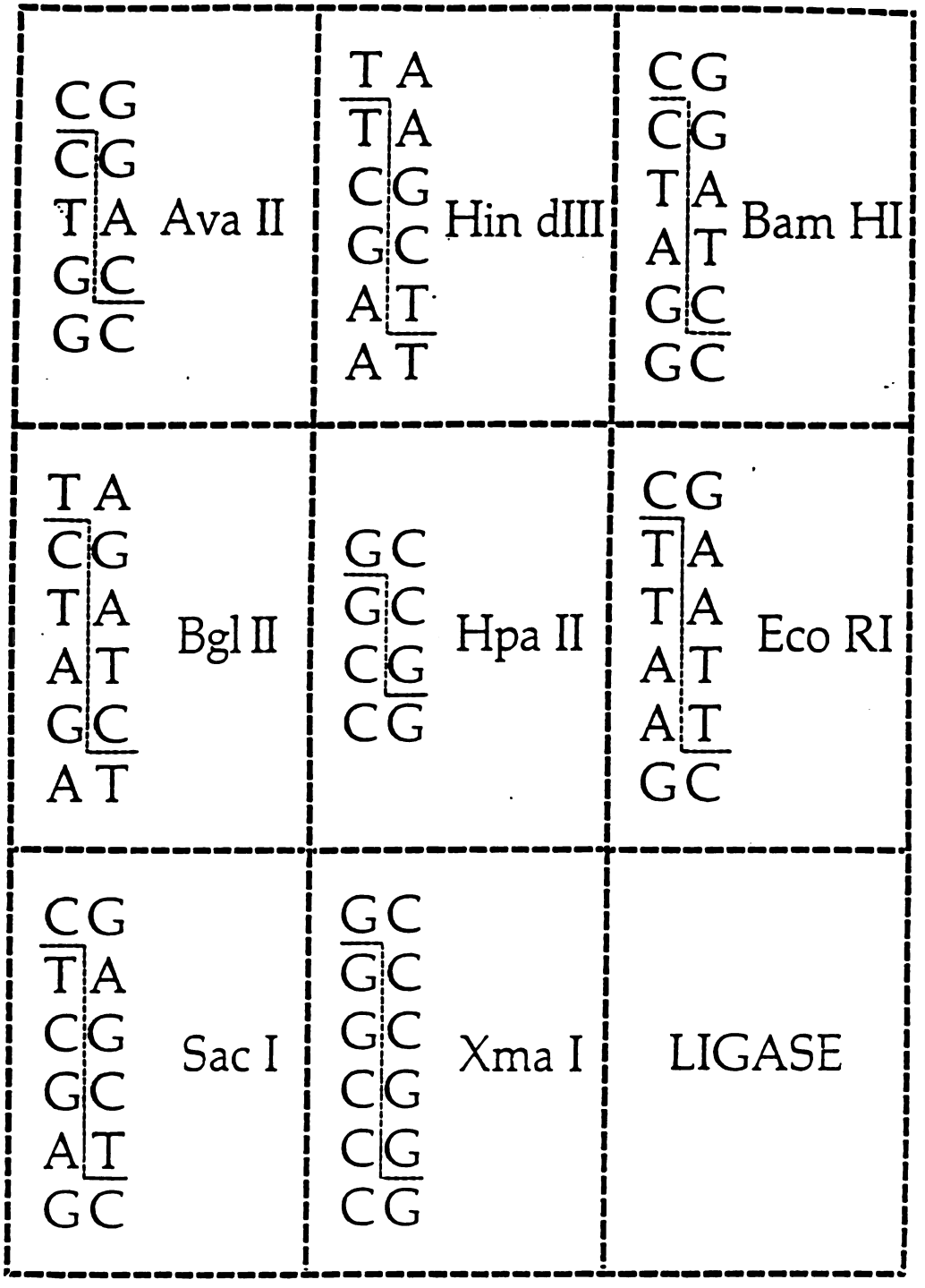
6. How do you determine which restriction enzyme to use?


PLASMID
= ampicillin resistance
= kanamycin resistance
= tetracycline resistance
= plasmid replication

CELL DNA

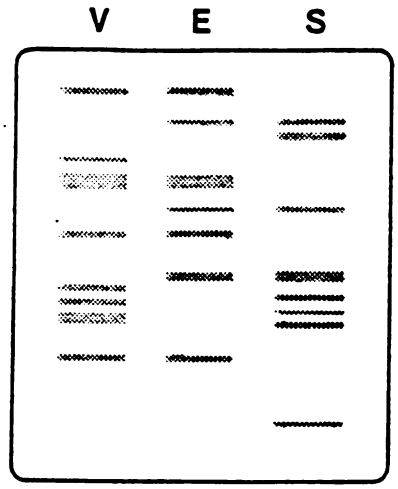


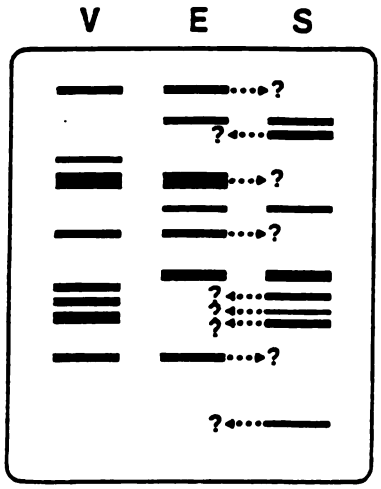
RESTRICTION ENZYMES



DNA Fingerprint Comparison 1

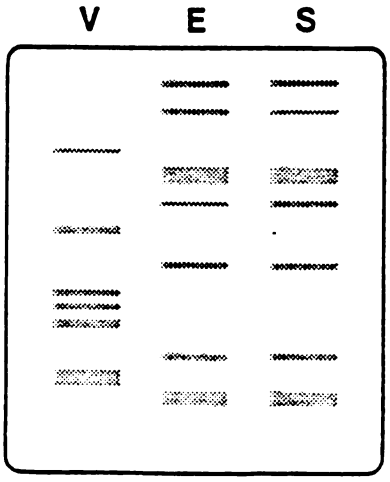
ANALYSIS OF DNA FINGERPRINTS IN FORENSIC TESTING

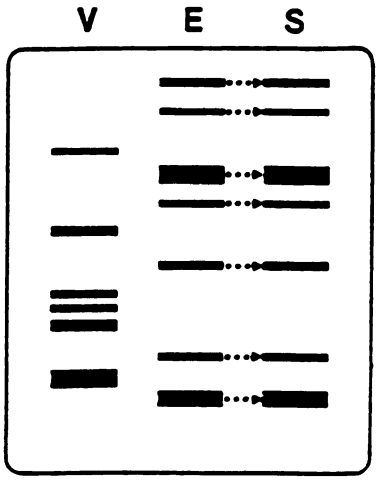




Answer:

NO MATCH





Answer:

MATCH

LEGEND

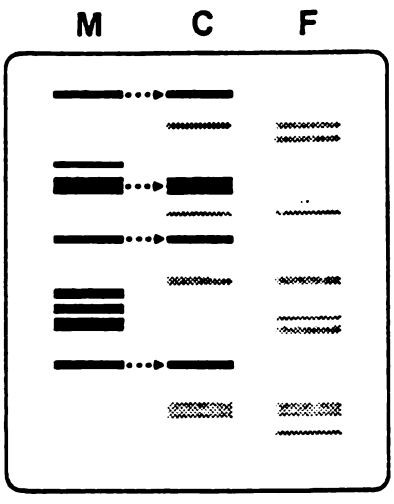
V = Victim

E = Evidence

S = Suspect

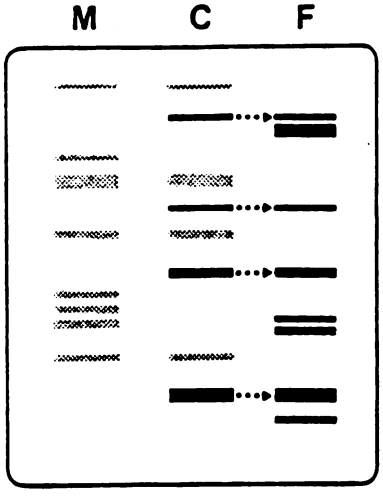
DNA Fingerprint Comparison 2

Analysis of DNA Fingerprints in Paternity Testing



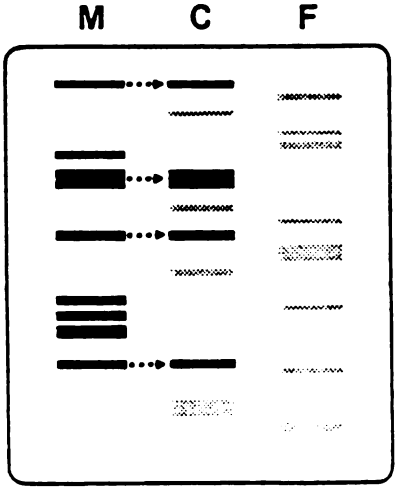
Identify maternal bands in child

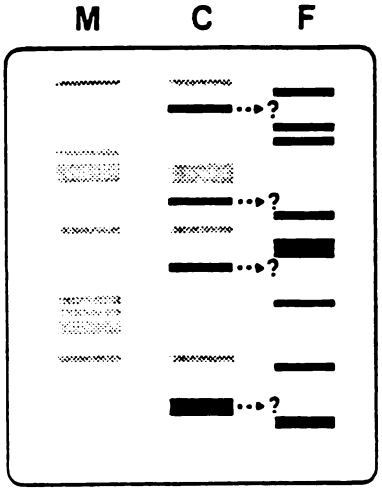
Remaining bands in child must come from the father



Do paternal bands in child derive from father?

Answer: YES
Paternity established





Answer: NO
Paternity disproved

Figure 6

DNA CARDS

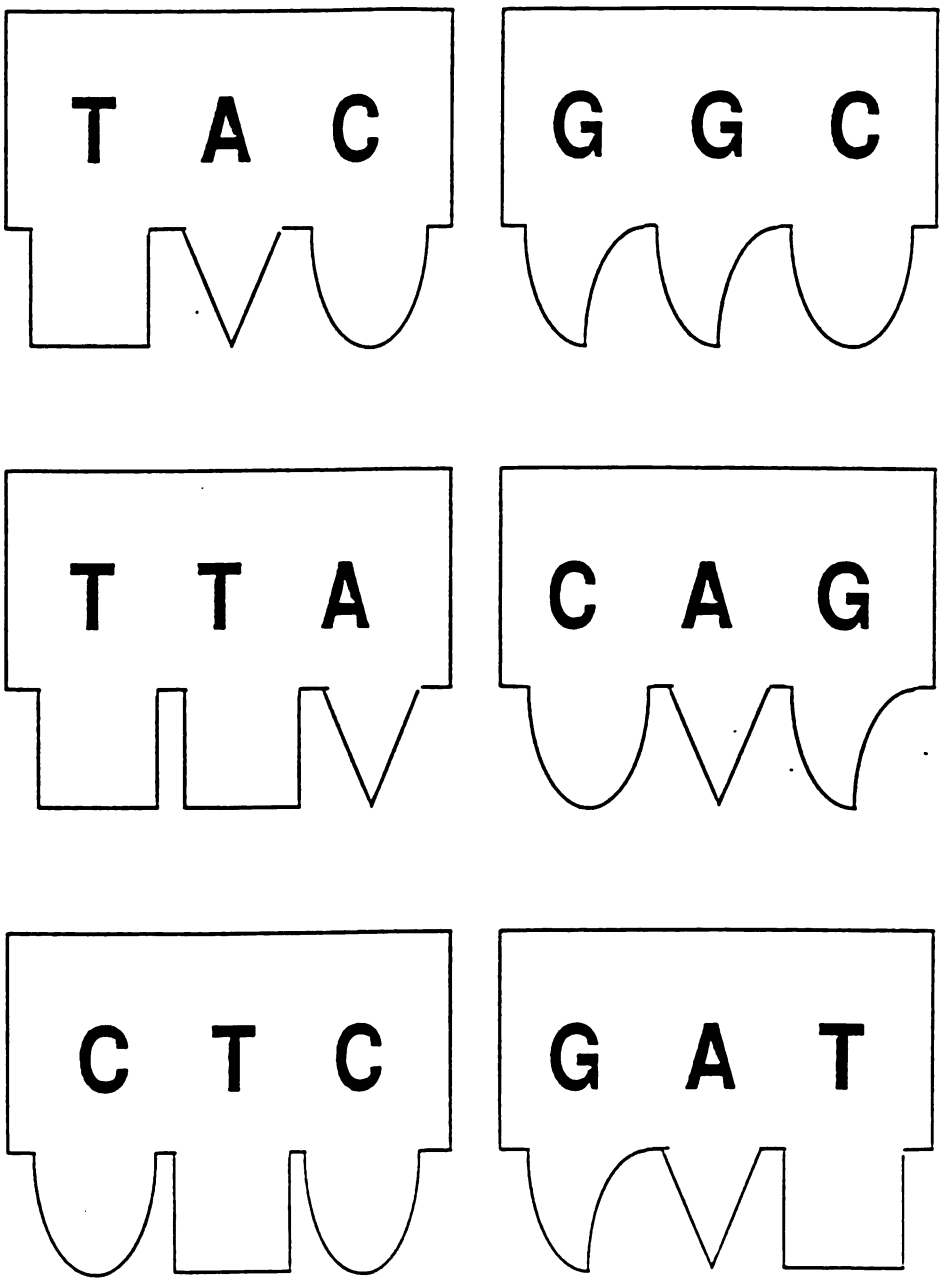


Figure 7

DNA CARDS

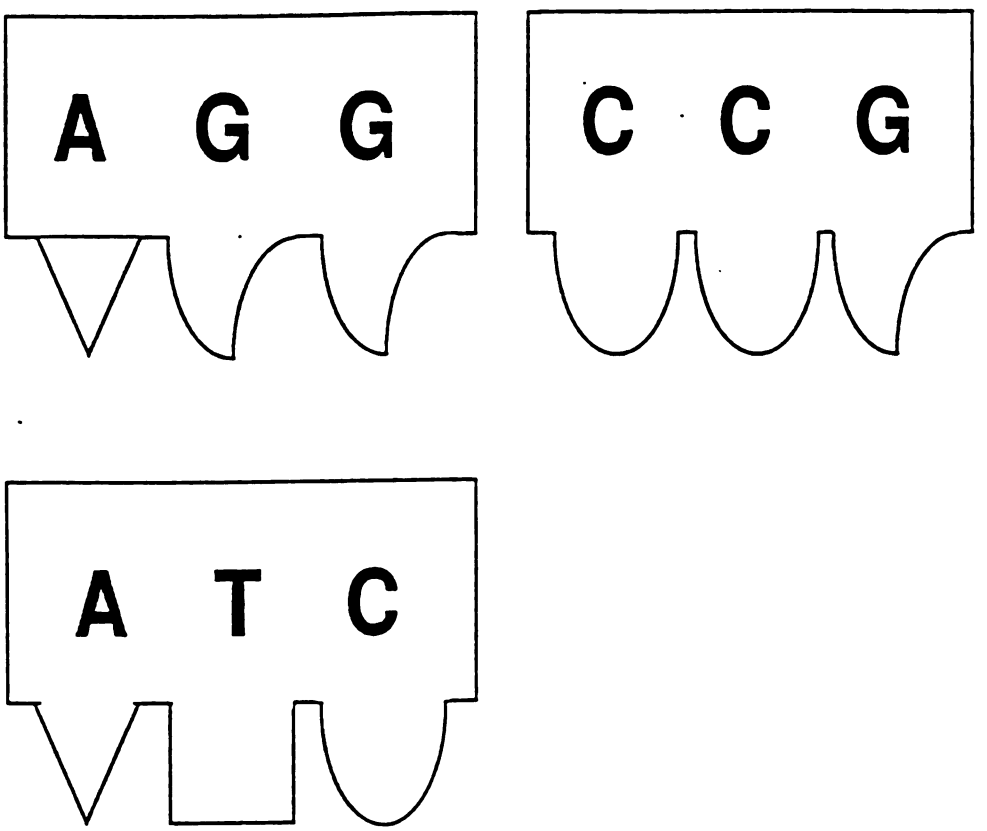
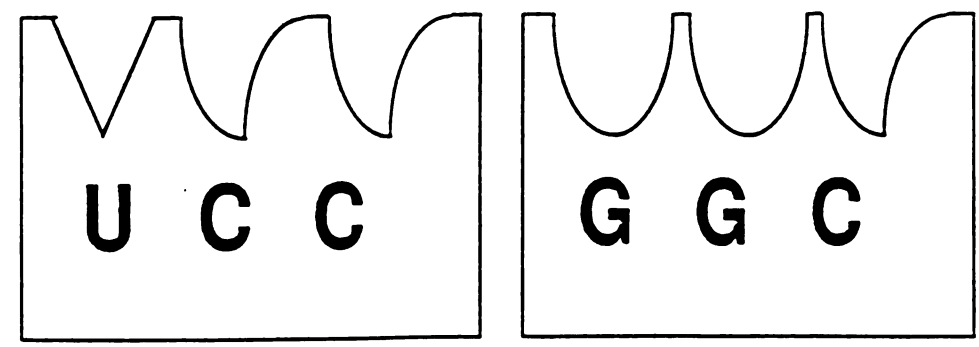


Figure 9

mRNA CARDS



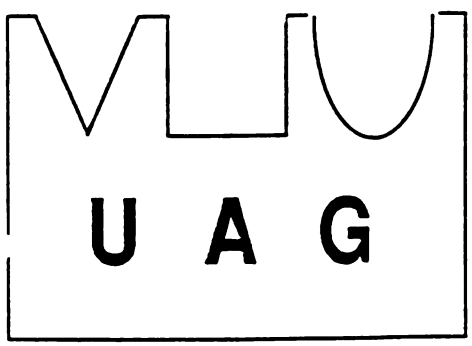


Figure 10

tRNA CARDS

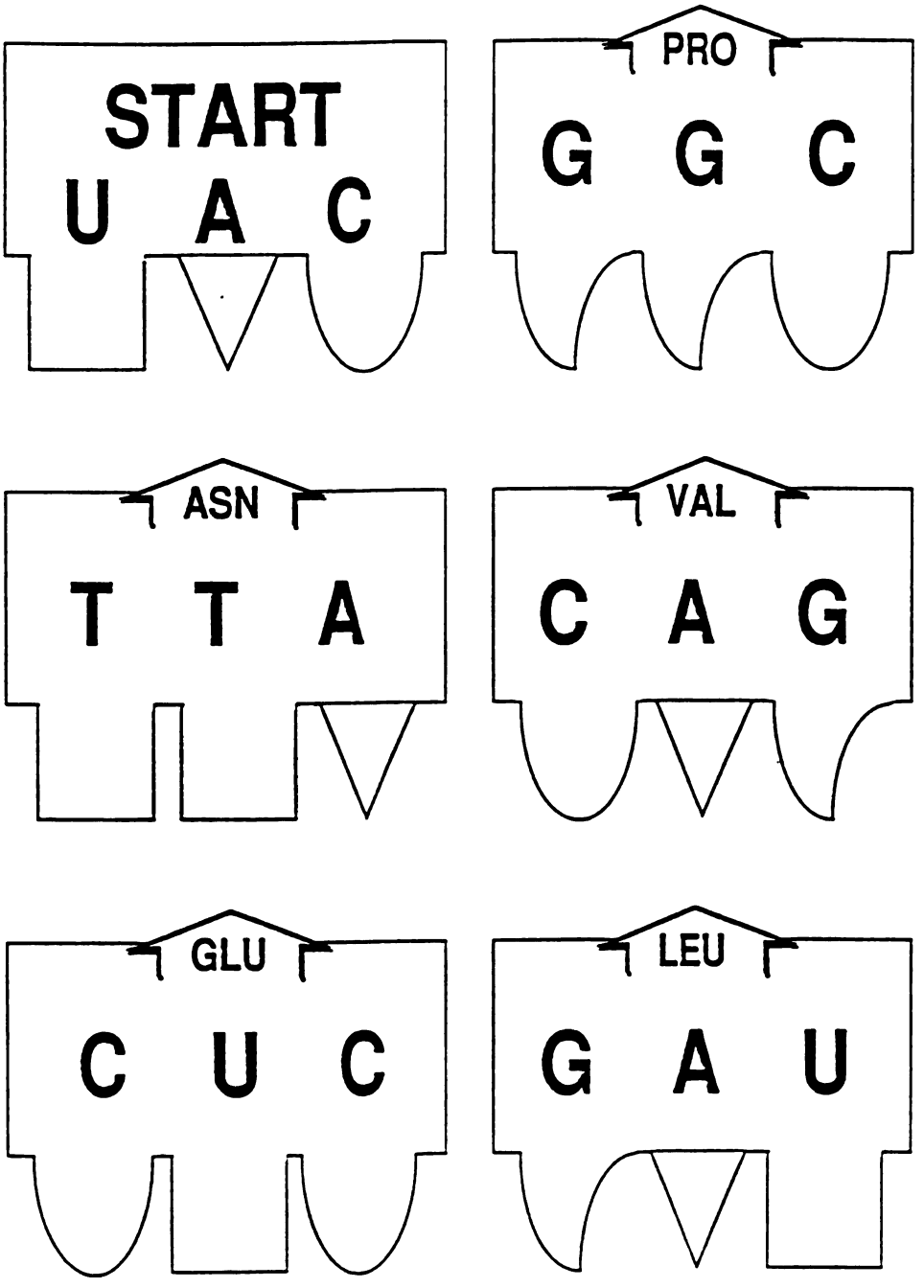


Figure 11

tRNA CARDS

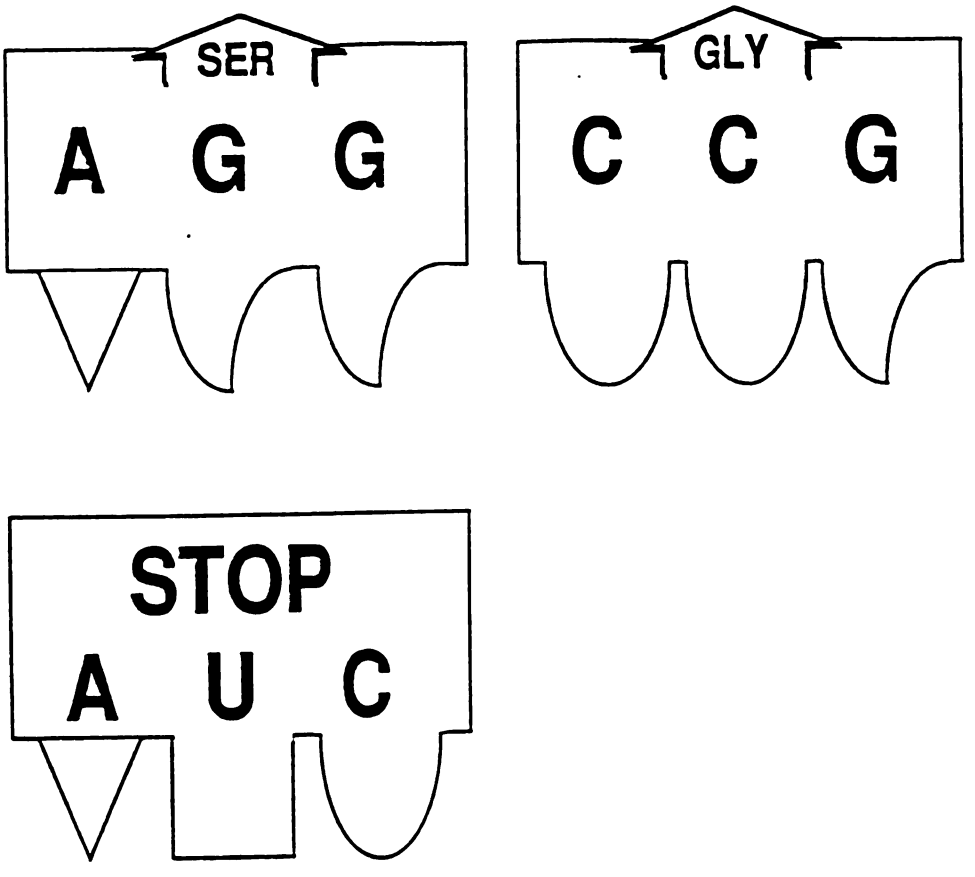
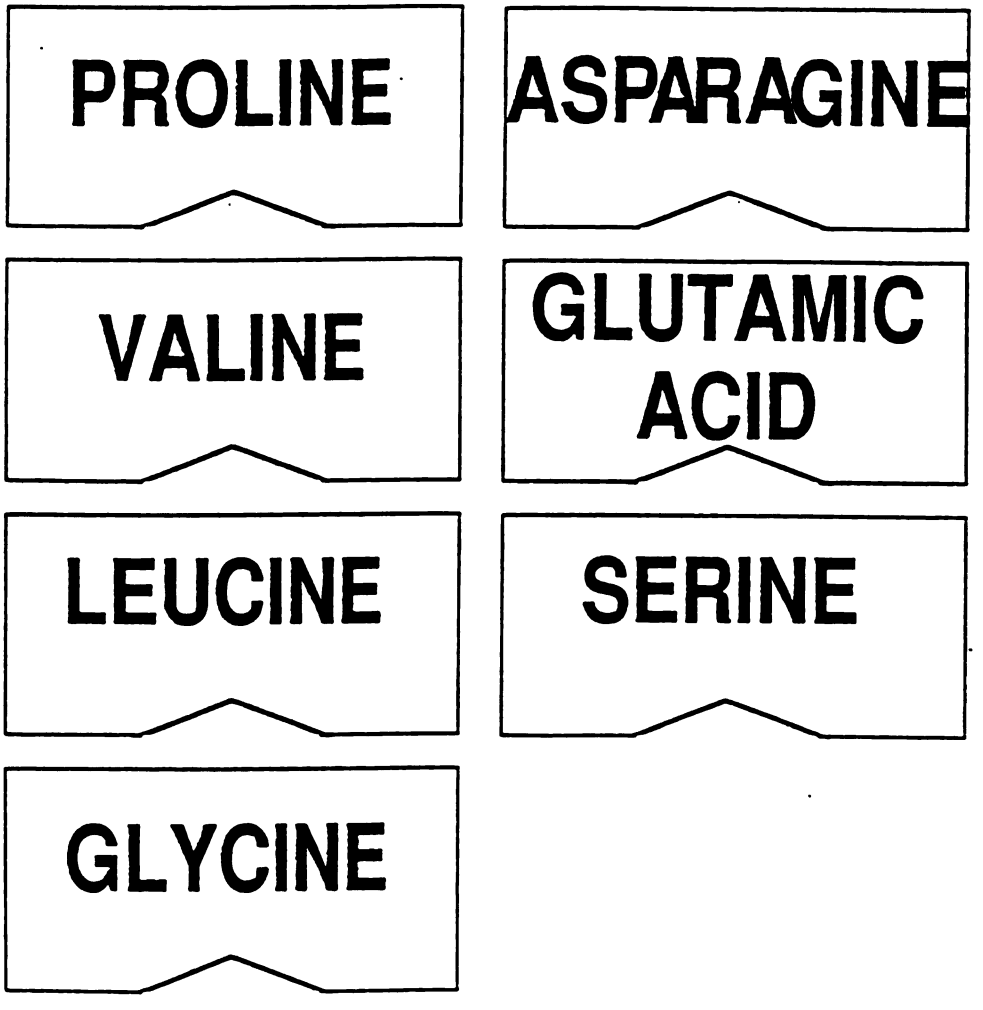


Figure 12

AMINO ACID CARDS



Name_____
Hour_____

DNA SCIENCE QUESTIONNAIRE

Please answer the following survey using your knowledge of each subject.
Using the scale 0 - 5 rate each subject on how well you know the material.
0 = no knowledge and 5 means great knowledge.

Rate	**Subject**
_____	The structure of DNA
_____	The function of DNA
_____	Relative size of the DNA molecule
_____	Protein Synthesis (the process of building proteins)
_____	Gel Electrophoresis
_____	Making or reading Restriction Enzyme Maps
_____	Recombinant DNA
_____	PCR (polymerase chain reaction)
_____	DNA Sequencing
_____	DNA Fingerprinting

Biology I

Name_____
Hour_____

DNA Pretest

Please answer the questions to the best of your ability. Don't worry about writing a wrong answer, but please **do not** leave any blank.

1. As you look around the room, you see a variety of hair color. Why do you see predominantly dark hair though?

2. What is DNA's shape and where can it be found?

3. What is the function of DNA?

4. Is it possible to remove DNA from a cell without damaging it?

5. Can DNA be seen under a microscope?

6. Is it possible for two people to have the same copy of DNA?

7. Does your liver cell have the same copy of DNA as your heart cell?

8. Does a heart cell contain the information about an individuals hair color?

9. Is it possible for a scientist to compare the DNA of one individual with another?

10. Look around the room and see the different characteristics people possess. Different sexes, hair color, height, eye color, skin color and so on. Yet all of us have two arms, two eyes, one mouth, ten fingers etc. Can you begin to explain why this is possible?

11. Who are Watson and Crick?

12. DNA is responsible for generating specific traits. Explain the process by which DNA actually aids the cells in consequently producing the trait.

Name_____
Hour_____

Recombinant DNA Post Test

Define the following words:

1. Recombinant DNA-

2. PCR (polymerase chain reaction)-

3. Restriction Enzymes-

4. Plasmid-

5. Human Genome-

Short Answer:

6. What is the general shape of DNA and specifically where can it be found?

7. Please compare and contrast DNA and genes.

8. How does RNA and DNA differ? (be sure to mention bases, whether it is double or single stranded and possible places it can be found)

9. What end result is made possible by the process of DNA replication?

True and False

_____10. Is it possible to remove DNA from a cell without damaging the molecule?

_____11. Insulin is a valuable product made from recombinant DNA.

_____12. Palindromes are "chemical scissors" used for cutting desired sections of sequences from a DNA strand.

_____13. It is still not possible for a scientist to compare the DNA of one individual with another.

_____14. A liver cell has the same copy DNA within the nucleus as a heart cell.

In The space below, Please draw a short section of DNA while labeling the following structures: Bases, Phoshate groups, Deoxyribose sugars, adenine, thymine, guanine and cytosine.

MATCHING:

_____1. Vector

_____2. Plasmid

_____3. Restriction Site

_____4. Human Genome

_____5. Transgenic Organism

_____6. Gene Splicing

_____7. Electrophoresis

_____8. clones

_____9. DNA fingerprinting

_____10. PCR

_____11. Palindrome

_____12. DNA sequencing

_____13. Restriction Enzyme

_____14. Insulin

A. a process of making more copies of a DNA strand.

B. a circular piece of DNA in a bacterium

C. a process to sort DNA fragments.

D. used to transfer DNA from one organism to another.

E. Madam I'm Adam

F. used to figure out the order of base pairs in a gene.

G. the exact place where DNA is cut by enzymes.

H. the rejoining of cut DNA sequences

I. a valuable product made from recombinant DNA.

J. Identical cells descending from one original cell

K. a bacterium possessing the insulin gene.

L. the total sum of all the genes in one human cell

M. a process used to separate DNA fragments creating patterns used to compare organisms of the same species.

N. "chemical scissors"

GEL ELECTROPHORESIS LAB

Multiple Choice: Choose the best answer

_____1. In order to make a gel, the solution was brought to a boil and then cooled slightly before pouring it into the gel tray. What apparatus was used to form the shape of the gel?

a. electrodes and a comb b. a comb and tape

c. micropipette and buffer d. buffer and electrodes

_____2. What was used to add the dyes to the wells?

a. a comb b. an Erlenmeyer flask

c. a micropipette d. a beaker

_____3. If a dye travels towards the cathode, the dye is said to be:

a. positive b. negative

_____4. Which dye is molecularly bigger?

a. orange G b. bromophenol blue

c. phenol red d. xylene cyanol

_____5. Which dye is positively charged?

a. pyronin Y b. phenol red

c. orange G d. xylene cyanol

_____6. Which dye was NOT included as an unknown:

a. orange G b. phenol red

c. pyronin Y d. bromophenol blue

_____7. If orange G travels through the dye at a faster speed than crystal violet, then orange G is :

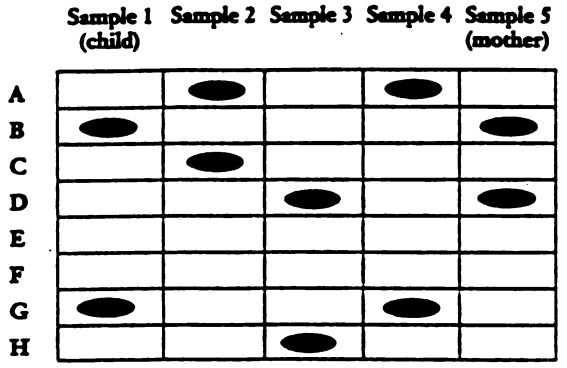
a. wetter than crystal violet b. bigger than crystal violet

c. more colorful than crystal violetd. smaller than crystal violet

APPLYING DNA FINGERPRINTING

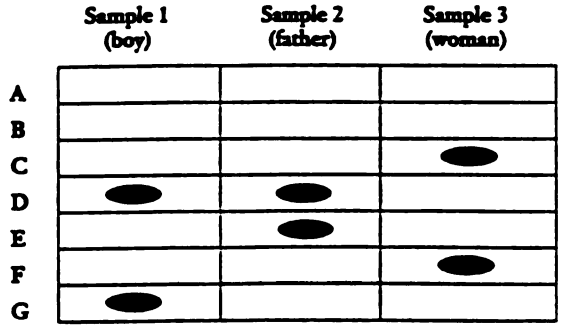
1. Mr. Jones was killed in an automobile accident. Afterwards, a former girlfriend filed a paternity suit claiming that Mr. Jones was the father of her child, and thus she was entitled to the man's estate. Keeping in mind that individuals receive half their DNA from their mother and half from their father, which sample of DNA would have to be from Mr. Jones if he were the child's father? (See Figure 2.) Explain your answer.

Figure 2

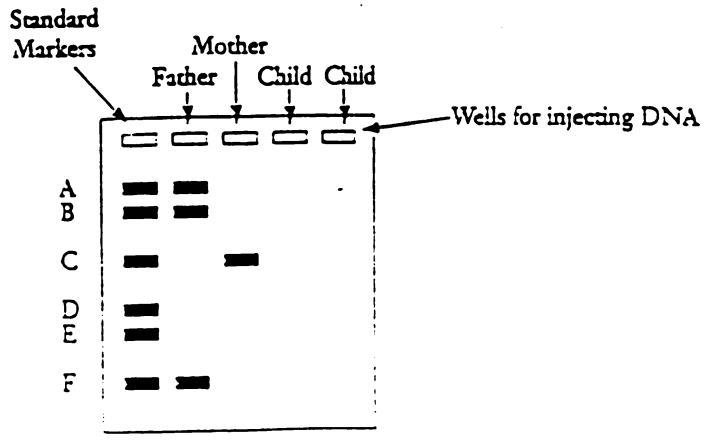


2. A boy moves to another country to live with his father. Years later, the boy decides to move back to live with the woman who reared him. Immigration officials suspected that the boy was the woman's nephew, not her son, and thus not legally entitled to residency. Based on the DNA fingerprints, is it possible that she is the boy's mother? (See Figure 3.) Explain your answer.

Figure 3



3. Below is a drawing of a hypothetical gel. Included on the gel is the banding pattern of the mother and father of two children. Draw what the pattern of bands for each of the children might look like. Indicate the possible parental source using ♂ for the male (father) and ♀ for the female (mother).



DNA UNIT TEACHER SURVEY

Dear Fellow Biology Teachers,

I would greatly appreciate your help if you could fill out this survey regarding the unit on DNA biotechnology that you are currently using in your biology class. Please take a few minutes to jot down some comments. These will be very helpful in the presentation of my thesis. If you have any questions, please don't hesitate to ask.

1. To begin, was using this unit a learning process for you as well as the students? Please comment.

2. Do you feel that the unit is at the appropriate level for the students?

3. Is the unit too lengthy: do you feel it is taking away too much time from our curriculum?

4. What labs (if any) do you think worked especially well or maybe didn't work at all. Keep in mind the concept of the lab: did the student gain an idea or concept from the lab or activity?

5. Will you use this unit again next year?

6. What things could you do to improve the unit?

7. Do you think any of the lab activities caused students to use their critical or higher thinking skills?

8. Do you feel it is important for a teacher to remain as current as possible in terms of teaching strategies and content information?

Thank You Very Much!!

Chris Forbush

WORKS CITED

Beardsly, Tim. "Pointing Fingers". Scientific American. Mar, 1992. 26-27.

Beck, Isabel. "Reading and Reasoning". The Reading Teacher. May 1989. 76-82.

Brown, William. "DNA Fingerprinting Back in the Dock". New Scientist. Mar. 1993. 18-24.

Culver, Kenneth W. "Splice of Life". Sciences. Feb. 1993. 18-24.

Darnell, James. Harvey Lodish, David Baltimore. (1990). Molecular Cell Biology Second Edition. New York. Scientific American.

Ezzell, Carol. "Closing in on the Lou Gehrigs Disease Gene". Science News. Jan. 2, 1993.

Franklin-Barbjosa, Cassandra. "DNA Profiling: The New Science of Identity". National Geographics. May, 1992. 112-124.

Jaroff, Leon. "Happy Birthday, Double Helix". Time. March,1993. 56-59

Klug, William, Michael Cummings. (1983). Concepts of Genetics. Ohio. Merrill.

Macklem, George, Karen Pawloski, Sarah Lintemuth, Andrea VonTom, Laurie Kaguni. "Especially for Teachers Section: Now that we have the DNA , what do we do with it"? Natural Science. Michigan State University. East Lansing, Michigan. Vol VI, No.1, 1992.

Micklos, David, Greg Freyer. (1990). DNA Science: A First Course in Recombinant DNA Technology. Carolina Biological Supply.

Miller, G Tyler. (1992). Living in the Environment. California. Wadsworth Inc.

Pawloski, Karen. (1996). Forensic Science in the High School Classroom. Michigan State University. East Lansing, Michigan.

Rasmussen, Alison, Robert Matheson III. A Sourcebook of Biotechnology Activities. 1990. Expert Graphics. Virginia.

Rasmussen, Trudy, Chris Forbush. "Especially For Teachers Section: The Isolation of DNA from Onion Tissue". Natural Science. Michigan State University. East Lansing, Mishigan. Vol V, No 2. !991-1992.

Roberts, Leslie. "DNA Fingerprinting Back in the Dock". New Scientist. Mar. 6 1993. 14-15.

Science Kit and Boreal Laboratories. "DNA Fingerprinting". New York.

Science Teacher. "Making a Recombinant Plasmid". !987. 44-48.

Stroh, Michael. "DNA Solves AIDS Epidemiological Whodunit". Science News. May 23, 1992.

Sulfian, Jane. "Forensic Use of DNA Tests". British Medical Journal. July 6, 1991.

United States. The National Commission on Excellence. A Nation at Risk: The Imperative for Education Reform. April 1993.

University of Michigan. "North Central Association of Colleges and Schools". 1993.

Yager, Robert and John Penick. "Resolving the Crisis in Science Education: Understanding Before Resolution". Science Education. Jan. 1987.