

Teacher Notes

Introduction

This is a simulation of the process of DNA fingerprinting. In this exercise, students will simulate:

- preparation of restriction fragments from samples of DNA, and
- separation of these fragments by gel electrophoresis.

These processes yield evidence regarding a crime, and students are asked to use the results of their simulated gel separations to infer which suspect's blood was left at the scene of the crime. News reports have publicized numerous crime events involving the use of DNA evidence. In spite of the publicity, much of the underlying science is not well understood by most people. As you will see, this is not a simple activity, but includes several advanced concepts. It assumes prior knowledge of the structure of DNA and its components, as well as some understanding of the enzymatic function of proteins. Finally, interpretation of results requires a careful analysis of some rules of logic.

Concepts in this Activity

The concepts emphasized in this activity include:

1. The use of restriction enzymes to prepare DNA fragments.
2. The use of gel electrophoresis to separate molecules of different sizes.
3. The meanings of the terms "sticky ends" and "blunt ends."
4. The reasons why multiple tests, using different restriction enzymes, are needed for DNA evidence.
5. The logic of scientific procedure (hypotheses may be rejected, but they may not be proven).
6. The use of statistical analysis to estimate the probability that a conclusion is correct.

Initiating the Activity

A crime scenario is described in the student instructions to introduce this activity. To save space, this scenario is not repeated here.

Summary of the Procedures

Students will work in pairs or groups. They will:

1. Simulate the use of restriction enzymes (EcoRI and HaeIII) to cut DNA samples into fragments.
2. Separate the fragments using simulated electrophoresis. This will yield the banding patterns which we call the "fingerprint," or more properly the "profile."
3. Compare the fingerprint (profile) from the crime scene with those of three suspects.
4. Interpret the results, deciding which, if any, of the suspects might have left the blood at the crime scene.

TxCETP Course Component: DNA Fingerprinting, “Who Done It?”

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Advance Preparation

1. Review of Student Procedures.

Review the student procedures carefully. If you have not yet completed the activity, you will want to do a run through so that you are familiar with the concepts and materials.

2. Pairs or Groups.

Decide whether to have students work in pairs or groups. Using groups may decrease the amount of time required to manipulate materials--marking, cutting, taping, interpreting, etc.

If students are not comfortable working in groups, you may want to structure some group activities to facilitate their work. For example, you might assign roles: project leader, materials manager, record keeper, and reporter. If you use groups of four, you will want to assign two students to work on EcoRI and two to work on HaeIII.

Further, you might have your students practice social skills needed to complete this activity such as listening, sharing information, providing positive leadership and sharing the work load. You have probably noted that accomplishing these behaviors requires more than telling students to do it. Some teachers have found it valuable to discuss these skills with students, and then ask them to describe ways they can demonstrate them. A third and very important step is for the teacher or member of the group to monitor the use of these skills, and to provide feedback to the group about their use of these social skills.

Also, remember that your role as teacher in group learning is to monitor all groups, intervening to help them meet their goals. Some teachers have found it helpful in working with student groups to allow only one person in the group to ask questions, forcing an intra-group discussion prior to the asking of each question.

3. Time Requirements.

Students who understand the directions and concepts can carry this activity through the completion of the gels in one 50-minute period. An additional period will be needed to record observations and interpret data.

Preparing materials

1. Make the following copies:

For each student group, 2 sets of gel lanes with 4 lanes per set (crime scene lane, suspect #1 lane, suspect #2 lane, suspect #3 lane). A master for each lane is provided. Each lane may be copied on a single 8.5x11" sheet of paper. Sets of four lanes will be taped together edge to edge to form a complete gel.

Make two copies of the DNA sample sheets for each pair or group. They must be printed on 17x11" paper, preferably on a color printer. Since students will use HaeIII on one sheet of samples and EcoRI on the other, there is some chance that the samples might become mixed.

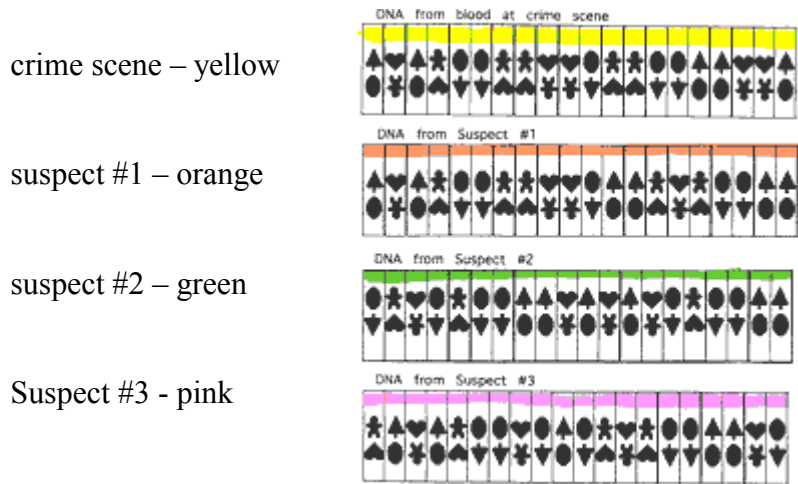
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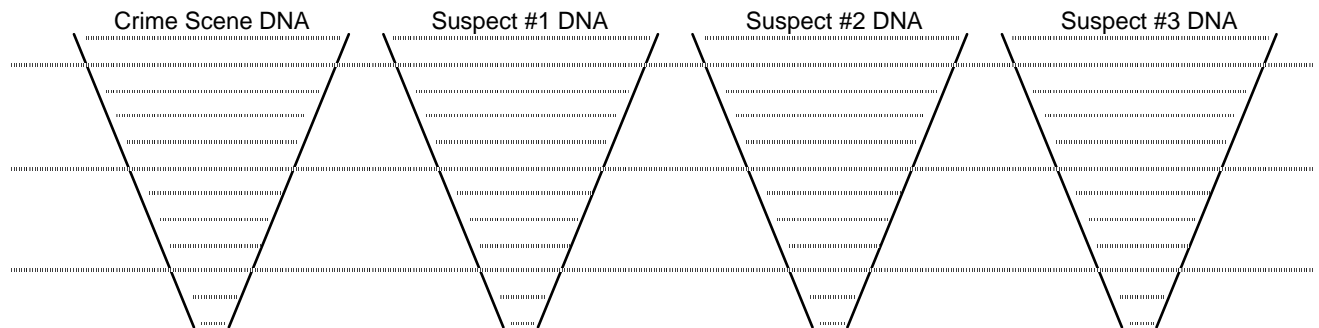
Therefore, you may want to run half of these on white and half on a lightly colored paper, and supply one white and one colored page to each pair or group.

Copies of the restriction enzyme templates. Two master copies are provided: one for the EcoRI enzyme, and one for the HaeIII enzyme. Run each of the two masters on transparency material (heavier is better). Make sufficient copies of the enzyme templates to provide one copy of each enzyme for each pair or group. Since they are small and easily lost, a few extra copies should be available.

2. If you don't have a color printer, code the DNA sample sheets with a highlighter (or crayon) by drawing a colored line on each sample. This will ensure that students will be able to distinguish between fragments from the crime scene and from the suspects once they have been cut apart. You may choose to have students mark these materials if you have enough markers. For consistency with the student directions, use the following colors:



3. Assemble the electrophoresis gel blanks. Four lanes are lined up side by side as indicated in the student instructions, and taped together. To save time, you may wish to have students assemble these in advance.

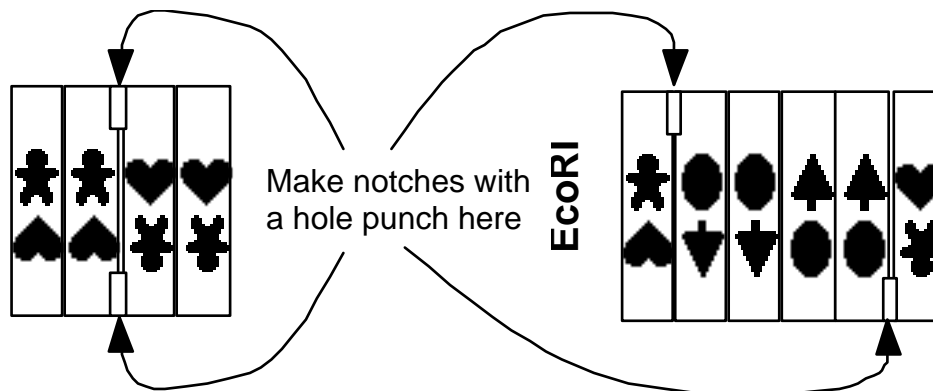


4. Secure sufficient pencils, scissors & cellophane tape for each group to operate smoothly.

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5. Prepare the restriction enzymes templates. Cut the transparencies of enzymes into small rectangles. For ease in marking the cutting sites and patterns (see student procedure) it will be a good idea to use a hole-punch to punch a hole at the cut site on either side of the enzyme as indicated below.



Additional Hints and Expansion

1. You may wish to extend this activity and materials into a courtroom simulation. That might provide broader appeal to students and give them opportunity to participate in role-playing activities (lab technician who analyzed the DNA samples, police officer who collected the blood samples, prosecutor who must explain to the jury what the test results mean, etc.). Perhaps teachers and classes in social studies would like to team up to examine the law, courtroom procedures, etc., in cases involving DNA evidence.
2. It frequently happens in running this exercise that students make mistakes, in identifying the exact cut sites of the enzymes, in carrying out the physical cutting, or in positioning the fragments on the gel lanes. Analogous mistakes are sometimes made by scientists and technicians in labs carrying out real restriction fragment analysis. Your students may wish to discuss whether it should be required that any DNA evidence used in a trial must be double-checked by being run independently in at least two separate labs.
3. After completing this exercise, students will probably recognize that interpretation of DNA evidence is more complex than most people realize. Given the increasing popularity of DNA evidence in trials, should it be required that all lawyers and judges receive training in molecular biology to ensure that DNA evidence is used honestly and fairly? Should prospective jurors be questioned about their understanding of DNA, to ensure that they can interpret the evidence fairly?
4. This exercise offers several possibilities for cross-curricular expansion, in addition to those mentioned above. For example, chemistry and physics material can easily be incorporated into this exercise. What are gels made of? What sort of structure do they possess which allows DNA fragments to move through them? Why are DNA fragments electrically charged? Why was it specified that a direct current must be applied to the gel to separate the

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fragments? Why couldn't an alternating current be used? Connections may also be made to mathematics classes. How can we describe the geometry of the DNA molecule? What is a double helix? What do we mean when we say that there is a certain probability that restriction fragment patterns from different individuals might accidentally match? How can this probability be calculated? Why do multiple tests using different enzymes decrease the probability of an accidental match?

Background

Fingerprinting vs. Profiling. "DNA Fingerprinting" is the term commonly used in media and conversation, but scientists refer to this technique by the more correct term, DNA "Profiling." By its nature, a "fingerprint" is believed to be unique to an individual. Thus an identified fingerprint at a crime site is inferred to mean that the individual touched the surface where the print was found. For any DNA profile there always exists a probability that there might be two or more individuals with the same profile. This probability is virtually 100% in the case of identical twins, but has a certain finite probability for any two individuals in a population. These probabilities are calculated, based on estimates made by scientists of the occurrence of a particular DNA fragment in the population from which the DNA sample was drawn.

Restriction Enzymes. Students are sometimes curious about the strange names given to these enzymes. EcoRI received its name because it was the first restriction enzyme (number I) isolated from the R strain of *Escherichia coli*. HaeIII received its name because it was the third enzyme (number III) isolated from *Haemophilus aegyptius* (strain designations are not used for *Haemophilus* species). More than 300 different restriction enzymes have now been discovered and characterized. A list of some of the most frequently used of these enzymes is provided in an appendix.

Leukocytes. You may wish to ask the students why the scenario emphasized that the crime scene DNA was prepared from leukocytes (white blood cells) collected from the crime scene blood. Why not use red blood cells, which are far more numerous in any blood sample? The reason is that in all mammals (including humans), the red blood cells contain NO DNA. By the time red blood cells are released into circulation, their chromosomes and the nuclei in which the chromosomes are found, have been extruded from the cells. Without the DNA-containing chromosomes, red blood cells cannot divide and have only limited ability to repair themselves, and will gradually deteriorate. But by the time any red blood cell is removed from circulation by filtering in the liver, it will have been replaced by another red cell, also lacking DNA.

Interpretation of Results

Now we turn to the interpretation of the results. What conclusions can we draw from the results of the two simulated digestions and electrophoretic separations? Most students will infer that suspect #2 is guilty. When asked to explain or justify this conclusion, students will point out that following digestion with EcoRI, only suspect #2's DNA restriction pattern matches that of the crime scene blood; therefore suspect #2 is the burglar. But is this a valid conclusion?

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Let us look carefully at our experimental results. Following digestion with HaeIII, it should be obvious that Suspect #3 cannot be the burglar; the restriction fragments from Suspect #3's DNA clearly do not match those from the crime scene. But BOTH Suspect #1 AND Suspect #2 yield restriction fragment patterns matching the pattern from the crime scene. We cannot distinguish between them, based on this information. When we do another digestion, using EcoRI, we obtain a fragment pattern from Suspect #1 which clearly does not match that from the crime scene. Thus we are able to eliminate Suspect #1 from consideration. Only Suspect #2 remains -- but does this prove he is the burglar?

Contrary to most students' expectations, this does NOT prove that Suspect #2 must be the burglar. It only demonstrates that we have not proven that suspect #2 is not the burglar. Suppose that we repeated the digestion procedure with still another enzyme -- perhaps we would now obtain a fragment pattern from Suspect #2 that would not match that from the crime scene. Now we could conclude that Suspect #2 is not the burglar after all, even though our first two digestion procedures produced apparent matches between his DNA and that from the crime scene. But what if this third digestion again produced a fragment pattern from Suspect #2 which did match that from the crime scene -- have we now proven that he is the burglar? No, we still have not proven that he is the burglar. We have only failed to prove that he is not the burglar. This is a subtle point, one which students, teachers, and scientists sometimes fail to grasp.

Many people suppose that in testing hypotheses, only two possibilities exist: we either prove or we disprove the hypothesis. In fact, there are three possibilities: we may prove the hypothesis, or we may disprove the hypothesis, or we may fail to disprove the hypothesis. Failure to disprove the hypothesis is NOT the same as proving the hypothesis. In fact, in experimental science, it is most often impossible to prove a hypothesis (although absolute proof can be obtained in some mathematical systems). We can only disprove or fail to disprove the hypothesis. Returning to our restriction fragment patterns, we recognize that a mismatch between a suspect's pattern and the crime scene pattern disproves the hypothesis that the suspect is the burglar. But a match between a suspect's pattern and the crime scene pattern only means that we cannot exclude the possibility that the suspect is the burglar; we have failed to disprove the hypothesis that the suspect is the burglar. And in fact, no matter how many times we repeat the digestion and obtain a match between the suspect's pattern and the crime scene pattern, we can never prove that he is the burglar, because it can always be argued that if we tried just one more enzyme, we would get a mismatch.

Of course, we would like to achieve more certainty. For many human activities, we need more certainty than just failing to disprove a hypothesis. In a criminal case, as well as in many scientific investigations, we need to draw definite conclusions. We need to be able to make a stronger statement than merely saying that we cannot prove that a hypothesis is false. For this reason, statistical techniques have been developed which allow us to estimate how likely or how probable it is that a particular result or set of data might have occurred by chance. In other words, techniques are available which allow us to estimate that there is (for example) an 80% probability that chance alone might have produced data which fit our hypothesis, or a 95% probability that results in agreement with our hypothesis could not have arisen by chance alone (another way of stating this is to say that in only 5% of cases could the data have occurred by chance). This is still not the same as saying that it is 100% certain that a hypothesis is true, but at

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least it suggests how close we may be to certainty. In general, if statistical techniques indicate a 95% or greater probability that chance alone could not have produced the data, we say that the data support the hypothesis. Notice the language: we say the data support the hypothesis, not that the data prove the hypothesis.

By analyzing DNA samples from many individuals in a population, we can find out how frequently particular sequences occur. The more frequently a particular sequence occurs in the population, the more likely it is that the sequence might be found in any DNA sample. If we use restriction tests which recognize common sequences, we might get accidental matches quite often. If we know that a particular sequence occurs with a frequency of only 0.1% (one individual out of 1000 carries this sequence), then in a city of 500,000 people there might be as many as 500 people whose DNA includes that sequence. On the other hand, if we use restriction enzymes which recognize relatively rare sequences, and if we carry out not just one test but several, we can greatly decrease the likelihood of accidental matches. But suppose we calculate that the chance of an accidental match between DNA samples from two individuals is only 0.00001% (one chance in ten million of an accidental match). The population of the U.S. is more than 250 million people. This means that approximately 25 individuals in the U.S. have DNA sequences that would match in these particular digestions. If you were serving on a jury and were given evidence that this suspect is one of about 25 people whose DNA might have this pattern, would you think this evidence strong enough to send the suspect to prison? Obviously not every person in the U.S. population is a suspect. Should we ask instead how many people in the state or in the city where the crime occurred have this DNA pattern? Arguments of this sort supply the reason why evidence in addition to DNA profiles must be presented in a criminal case. Is there evidence that the suspect was in the vicinity when the crime was committed? Remember that not all 250 million U.S. residents are actually near the crime scene! Was any of the loot from the burglary found in the suspect's car or home? Are there additional pieces of incriminating evidence which lead a reasonable person to conclude that this suspect committed the crime?

After repeated digestions with different enzymes, each producing a match between the suspect's DNA and that from the crime scene, we might become very confident that he is the burglar. But we can never achieve absolute certainty, we can never prove that he is the burglar, because the possibility always remains that one more test would produce a mismatch. This exercise illustrates a general point about the testing of hypotheses: we can never achieve absolute certainty; we can never prove a hypothesis. We can disprove a hypothesis. We can fail to disprove a hypothesis. But failure to disprove does not equal proof. All our conclusions must remain tentative, subject to revision in light of subsequent discoveries. This tentativeness, this willingness to revise our ideas in light of additional information, is one of the hallmarks of true scientific procedure.

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