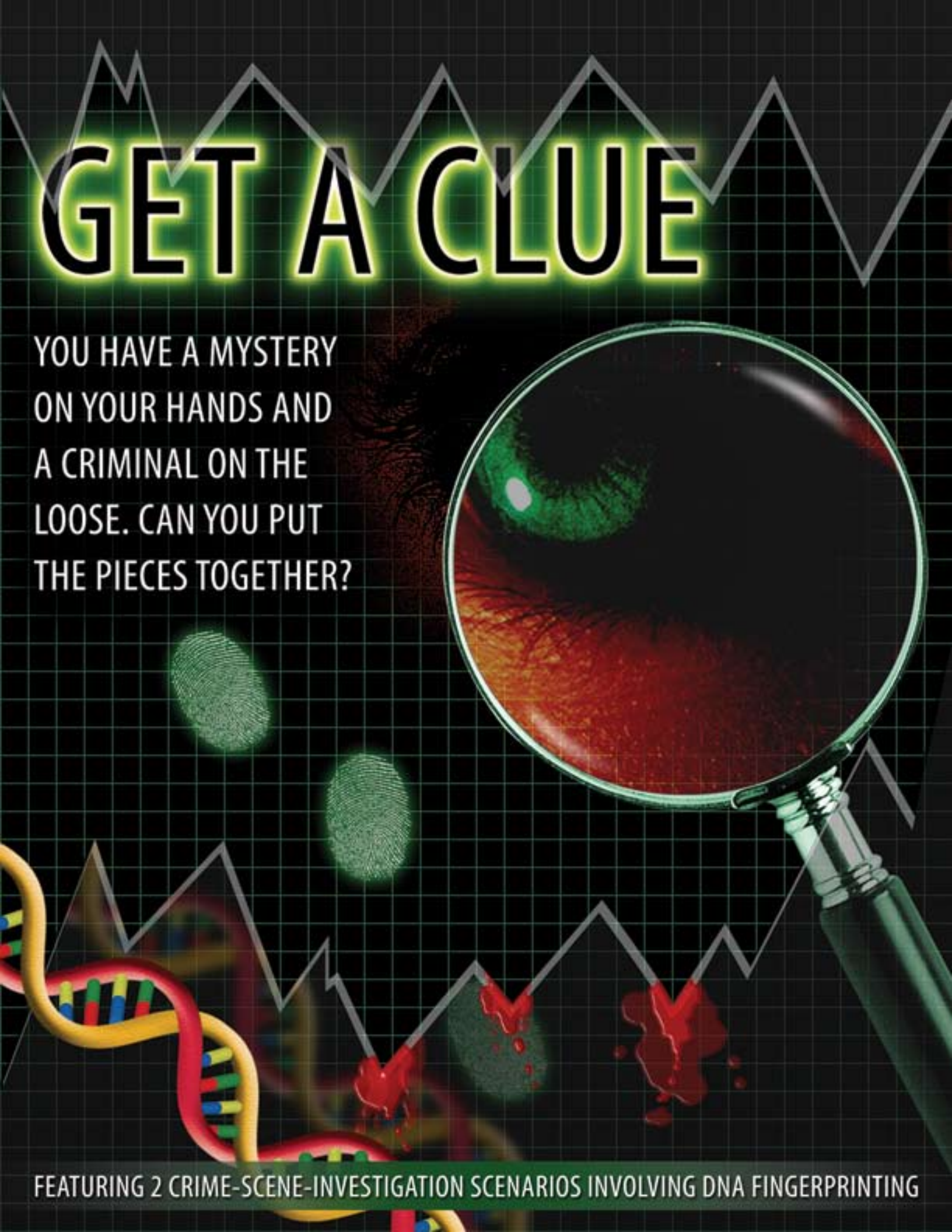
GET A CLUE

YOU HAVE A MYSTERY ON YOUR HANDS AND A CRIMINAL ON THE LOOSE. CAN YOU PUT THE PIECES TOGETHER?



FEATURING 2 CRIME-SCENE-INVESTIGATION SCENARIOS INVOLVING DNA FINGERPRINTING

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TABLE OF CONTENTS

KEY TERMS 5

ALIGNMENTS 7
The Key Components of the 5E Model 7
North Carolina Standard Course of Study 8
National Science Education Standards 11

INTRODUCTION 13
Background 13
Alternative DNA Fingerprinting Scenarios 14
The Process of DNA Fingerprinting 17
Restriction Fragment Length Polymorphisms 19
Pre-lab 20
Wet-lab 20
Post-lab/Additional Activities 21
Connection to Other Modules 24

PRE-LAB 25
Utilizing the 5E Instructional Model 26
Engagement Activity 27
Engagement Activity Scenario 1:
The Case of the Tempting Tiara 28
Engagement Activity Scenario 2:
The Case of the Irresistible iPods 31
Exploration Activity 34
The Evidence Report 35
Additional Information on Blood Types 35
After You Complete the
Fiber-Identification Activity 36
Instructions for Fingerprints Activity 36
Fiber Identification 37
Identification Using the Burn Test 38
Fingerprints 39
Fingerprints Data Sheet 40
Determining the ABO-Rh Blood Types
of Simulated Blood Samples 41
The Blood Evidence:
Determination of Blood Type of Suspect 42
Sources 43
DNA Evidence 44
Explanation/Elaboration Activity 49
Elaboration Activity 50
Figure 1: X-Ray Diffraction Photograph of DNA 51
Figure 2: DNA Structure 52
Figure 3: Milestones in Forensic DNA Analysis 53
Figure 4: How DNA Is Collected
by the Crime-Scene Investigators 54
Restriction Enzymes 55
KEY Restriction Enzymes 56
Gel Electrophoresis 57
KEY Gel Electrophoresis 58
Short Tandem Repeats (STRs) 59
Polymerase Chain Reaction (PCR) 59
Examples of STR 60
CODIS 60
Power of Discrimination 61
Calculating a DNA Profile Frequency 63
KEY Calculating a DNA Profile Frequency 64
Evaluation: Incriminating Evidence 65

WET-LAB 67
Wet-lab Engagement Activity 68
Agarose Gel Electrophoresis and
Visualization of DNA Fragments 73
Quick Guide for Forensic
DNA Fingerprinting Kit 75
Equipment Needed for Wet-lab 78

POST-LAB 79
Get a Clue Quiz Game Questions 80
KEY Get a Clue Quiz Game Answers 81

ADDITIONAL ACTIVITIES 83
DNA Restriction Enzyme and Probe Worksheet 84
KEY DNA Restriction Enzyme
and Probe Worksheet 85
The Ima Mystery Case 86
KEY The Ima Mystery Case 87
Taking Fingerprints Activity 88
Blood Typing: Practice Using Punnett Squares 91
KEY Blood Typing:
Practice Using Punnett Squares 93
A Crime on Campus 94
Enzyme Used: EcoRI 95
KEY Enzyme Used: EcoRI 96
Gel Electrophoresis Laboratory Report 97
KEY Gel Electrophoresis
Laboratory Report 98
Enzyme Used: BamHI 99
KEY Enzyme Used: BamHI 100
Gel Electrophoresis Laboratory Report 101
KEY Gel Electrophoresis
Laboratory Report 102
Quantitative Analysis of DNA Fragment Sizes 103
KEY Quantitative Analysis
of DNA Fragment Sizes 108

INTERDISCIPLINARY BRIDGES 113
Mock Trial 114
Integrating Forensics, Civics, and
World Literature: The Brothers Karamazov 116
Literary Crime Scenes 118
A Jury of Her Peers 119

KEY TERMS

Agarose gel — a semi-solid matrix formed by a polymer that creates an environment similar to a very densely woven spider web that enables molecules to be sorted by size (i.e., DNA fragments), shape, or electrical charge.

Blunt cut — a cut resulting from restriction enzymes that cut both strands of the target DNA at the same place.

Buffer — solution that stabilizes pH and provides ions to conduct electricity across a gel.

Cleave — to cut or separate.

CODIS — Combined DNA Index System — a federally maintained database used by law enforcement officials.

Comb — the plastic structure inserted in the acrylic gel tray to make the wells (the indentations) in the agarose gel.

DNA — deoxyribonucleic acid — the chemical molecule that is the basic genetic material found in most cells DNA is the carrier of genetic information from one generation to the next.

DNA polymerase — an enzyme that synthesizes a new DNA strand from a template strand and "proofreads" the new copy to ensure that it is a near perfect copy of the original or template DNA strand.

DNA fingerprinting — the technique of comparing RFLP's of different DNA samples obtained by sorting DNA fragments according to size using gel electrophoresis. Bands are compared with the control to determine which person's DNA matches the control DNA.

DNA fragments — DNA segments resulting when DNA is cut with a restriction enzyme. Fragments of different sizes (lengths) are produced.

DNA restriction analysis — used to help further our knowledge about the structure of DNA, for mapping and sequencing DNA, and also for DNA typing for identification purposes. Restriction analysis has three parts: DNA digesting, electrophoresis, and staining plus analysis. DNA fingerprinting utilizes DNA restriction analysis.

Digital micropipet — a basic tool of the biotechnologist that accurately measures liquid volumes in microliters (µl).

Electric field — electricity is used to move the DNA/protein through the gel matrix. When placed into an electric field, the charged molecules will migrate towards the opposite pole with the smaller fragments moving the fastest and traveling the farthest.

Evidence — anything that has been used ,left, removed, altered, or contaminated during the commission of a crime or other events under investigation.

Exclusion — when the DNA profile from a victim or suspect is inconsistent with the DNA profile generated from the crime scene evidence, the individual is "excluded" as the donor of the evidence.

Fingerprint — the unique pattern created by skin ridges created by skin found on the palm sides of fingers and thumbs.

Gel electrophoresis — the process that uses gels made of agarose or some other polymer to separate DNA fragments or proteins by size, charge, or shape using electricity to move the electrically charged molecules through the gel. As the DNA moves through the tangled pores of the agarose fibers, the smaller pieces move faster and the larger pieces more slowly.

Gel lanes — the paths the molecules travel through the gel from the wells to the opposite end of the gel.

Gene — a sequence of DNA that codes for a protein and determines a trait.

Human genome — the human genome is the complete DNA sequence, including all 46 chromosomes found in humans.

Hybridization — the binding of complementary nucleic acids.

Inclusion — when the DNA profile of a victim or suspect is consistent with the DNA profile from the crime scene evidence, the individual is "included" as the possible source of that evidence.

Inconclusive — inconclusive results indicate that DNA testing could neither include nor exclude an individual as the source of biological evidence. Inconclusive results can occur for many reasons: for example, the quality or quantity of DNA may be insufficient to

produce interpretable results, or the evidentiary sample may contain a mixture of DNA from several individuals (e.g., a sample taken from a victim of a gang rape).

Luminol — a chemical that is capable of detecting bloodstains diluted up to 10,000 times. Luminol is used to identify blood that has been removed from a given area. It is an invaluable tool for investigators at crime scenes that have been altered.

Microliter — (µl) a unit of measure used to measure liquid volume in molecular biology; 1000 µl = 1 ml.

Nucleotides — the four basic units that make up the DNA molecule. These are adenine (A), cytosine (C), guanine (G) and thymine (T).

Palindrome — a sequence of letters, words, or phrases that reads the same regardless of direction, for instance "Bob" or "madam." In reference to DNA, the sequence of nucleotides on one DNA strand is not a true palindrome. A DNA palindrome is found on a double strand of DNA whose 5' to 3' base pair sequence is identical on each strand. An example might look like this:

GAATTC
CTTAAG

Physical evidence — any object that can help explain an event under investigation. For example, physical evidence can establish that a crime has been committed, and sometimes it can provide a link between a crime and its victim or between a crime and its perpetrator.

Polymerase chain reaction (PCR) — a method used to make multiple copies of DNA in a laboratory setting.

Recombinant DNA technology — the techniques used to cut and create new combinations of DNA often from different organisms.

Restriction digest — the process of using any of the restriction enzymes that cut nucleic acids at specific restriction sites to produce fragments which are then known as restriction fragments.

Restriction enzymes — (restriction endonucleases) enzymes that act as "molecular scissors" to cut the DNA at a specific sequence (palindromic sequence) of nucleotides.

Restriction site — the specific sequence of nucleotides (palindromic sequence) that the restriction enzyme recognizes and "cuts" resulting in DNA fragments of different sizes.

RFLP (restriction fragment length polymorphism) — variation in the sizes of fragments produced when the DNA from different individuals is cut by one or more restriction enzymes. These polymorphisms are used as reference markers for mapping in relation to known genes or other RFLP loci.

Ridge characteristics — ridge endings , bifurcations, enclosures, and other ridge details, which must match in two fingerprints for their common origins to be establisned.

Staggered cut — the cleavage of two opposite strands of duplex DNA at points near one another by a restriction enzyme; useful for the creation of recombinant DNA molecules.

Southern blotting — a process in which DNA fragments on a gel are transferred to a positively charged membrane (a blot) to be labeled RNA or cDNA fragments.

Sticky ends — the single-stranded ends that result when restriction enzymes cut the DNA in an offset fashion, resulting in an end that has an overhanging piece of single stranded DNA. These single-stranded ends can anneal to other sticky ends that have complementary nucleotide sequences; helpful in producing recombinant DNA molecules.

STR — short-tandem repeats; micro satellites that contain 2-5 bases pair repeats.

VNTR — abbreviation for variable number of tandem repeats, sections of repeated DNA. Sequences found at specific locations on certain chromosomes; the number of repeats in a particular VNTR can vary from person to person; used in DNA fingerprinting.

Wells — the small, cup-like structures or indentations left in the agarose gel when the comb is removed. These wells will be filled with DNA or protein prior to electrophoresis.

The Key Components of the 5E Model

PHASE	WHAT THE TEACHER DOES THAT IS	
	Consistent with the 5E Model	Inconsistent with the 5E Model
ENGAGE 	• Creates interest • Generates curiosity • Raises questions • Elicits responses that uncover what students know or think about the concept/subject	• Explains concepts • Provides definitions and answers • States conclusions • Provides premature answers to students' questions • Lectures
EXPLORE 	• Encourages students to work together without direct instruction from teacher • Observes and listens to students as they interact • Asks probing questions to redirect students' investigations when necessary • Provides time for students to puzzle through problems • Acts as a consultant for students	• Provides answers • Tells or explains how to work through the problem • Tells students they are wrong • Gives information or facts that solve the problem • Leads students step-by-step to a solution
EXPLAIN 	• Encourages students to explain concepts and definitions in their own words • Asks for justification (evidence) and clarification from students • Formally provides definitions, explanations, and new labels • Uses students' previous experiences as the basis for explaining concepts	• Accepts explanations that have no justification • Neglects to solicit students' explanations • Introduces unrelated concepts or skills
ELABORATE 	• Expects students to use formal labels, definitions and explanations provided previously • Encourages students to apply or extend concepts and skills in new situations • Reminds students of alternative explanations • Refers students to existing data and evidence and asks "What do you already know?""Why do you think . . . ?"	• Provides definitive answers • Tells students they are wrong • Lectures • Leads students step-by-step to a solution • Explains how to work through the problem
EVALUATE 	• Observes students as they apply new concepts and skills • Assesses students' knowledge and/or skills • Looks for evidence that students have changed their thinking or behaviors • Allows students to assess their own learning and group process skills • Asks open-ended questions, such as "Why do you think . . . ?""What evidence do you have?""What do you know about x?""How would you explain x?"	• Tests vocabulary words, terms and isolated facts • Introduces new ideas or concepts • Creates ambiguity • Promotes open-ended discussion unrelated to concept or skill

(Trowbridge & Bybee, 1990), adapted by Biological Sciences Curriculum Study
Available online at http://science.education.nih.gov/supplements/nih1/diseases/guide/module3.htm

2005-06 North Carolina Standard Course of Study for Biology — Grades 9-12

*** Highlighted sections are objectives addressed in the Get A Clue module

Strands: Nature of Science, Science as Inquiry, Science and Technology, Science in Personal and Social Perspectives. The strands provide the context for teaching of the content Goals and Objectives.

Competency Goal 1:
The learner will develop abilities necessary to do and understand scientific inquiry.

Objectives

1.01 Identify biological questions and problems that can be answered through scientific investigations.
1.02 Design and conduct scientific investigations to answer biological questions.
- Create testable hypotheses
- Identify variables
- Use a control or comparison group when appropriate
- Select and use appropriate measurement tools
- Collect and record data
- Organize data into charts and graphs
- Analyze and interpret data
- Communicate findings

1.03 Formulate and revise scientific explanations and models of biological phenomena using logic and evidence to:
- Explain observations
- Make inferences and predictions
- Explain the relationship between evidence and explanation

1.04 Apply safety procedures in the laboratory and in field studies:
- Recognize and avoid potential hazards
- Safely manipulate materials and equipment needed for scientific investigations

1.05 Analyze reports of scientific investigations from an informed, scientifically literate viewpoint including considerations of:
- Appropriate sample
- Adequacy of experimental controls
- Replication of findings
- Alternative interpretations of the data

Competency Goal 2:
The learner will develop an understanding of the physical, chemical and cellular basis of life.

Objectives

2.01 Compare and contrast the structure and functions of the following organic molecules:
- Carbohydrates
- Proteins
- Lipids
- Nucleic acids

2.02 Investigate and describe the structure and functions of cells including:
- Cell organelles
- Cell specialization
- Communication among cells within an organism.

2.03 Investigate and analyze the cell as a living system including:
- Maintenance of homeostasis
- Movement of materials into and out of cells
- Energy use and release in biochemical reactions

2.04 Investigate and describe the structure and function of enzymes and explain their importance in biological systems.

2.05 Investigate and analyze the bioenergetic reactions:
- Aerobic respiration
- Anaerobic respiration
- Photosynthesis

Competency Goal 3:
The learner will develop an understanding of the continuity of life and the changes of organisms over time.

Objectives

3.01 Analyze the molecular basis of heredity including:
- DNA replication
- Protein synthesis (transcription, translation)
- Gene regulation

3.02 Compare and contrast the characteristics of asexual and sexual reproduction.

3.03 Interpret and predict patterns of inheritance.
- Dominant, recessive and intermediate traits
- Multiple alleles
- Polygenic inheritance
- Sex-linked traits
- Independent assortment
- Test cross
- Pedigrees
- Punnett squares

3.04 Assess the impact of advances in genomics on individuals and society.
- Human genome project
- Applications of biotechnology

3.05 Examine the development of the theory of evolution by natural selection, including:
- Development of the theory
- The origin and history of life
- Fossil and biochemical evidence
- Mechanisms of evolution
- Applications (pesticide and antibiotic resistance)

Competency Goal 4:
The learner will develop an understanding of the unity and diversity of life.

Objectives

4.01 Analyze the classification of organisms according to their evolutionary relationships.
- The historical development and changing nature of classification systems
- Similarities and differences between eukaryotic and prokaryotic organisms
- Similarities and differences among the eukaryotic kingdoms: protists, fungi, plants, animals
- Classify organisms using keys

4.02 Analyze the processes by which organisms representative of the following groups accomplish essential life functions including:
- Unicellular protists, annelid worms, insects, amphibians, mammals, non vascular plants, gymnosperms and angiosperms
- Transport, excretion, respiration, regulation, nutrition, synthesis, reproduction, and growth and development

4.03 Assess, describe and explain adaptations affecting survival and reproductive success.
- Structural adaptations in plants and animals (form to function)
- Disease-causing viruses and microorganisms
- Co-evolution

4.04 Analyze and explain the interactive role of internal and external factors in health and disease:
- Genetics
- Immune response
- Nutrition
- Parasites
- Toxins

4.05 Analyze the broad patterns of animal behavior as adaptations to the environment.
- Innate behavior
- Learned behavior
- Social behavior

Competency Goal 5:
The learner will develop an understanding of the ecological relationships among organisms.

Objectives

5.01 Investigate and analyze the interrelationships among organisms, populations, communities, and ecosystems.
- Techniques of field ecology
- Abiotic and biotic factors
- Carrying capacity

5.02 Analyze the flow of energy and the cycling of matter in the ecosystem.
- Relationship of the carbon cycle to photosynthesis and respiration
- Trophic levels — direction and efficiency of energy transfer

5.03 Assess human population and its impact on local ecosystems and global environments.
- Historic and potential changes in population
- Factors associated with those changes
- Climate change
- Resource use
- Sustainable practices/stewardship

Get A Clue
Correlation to the National Science Education Standards

The Teaching Standards	
Get A Clue Correlation	
Each activity in the module provides short-term objectives for students. There is a conceptual flow of activities and to help teachers plan a timeline for teaching the module. Use of this module helps teachers to update their curriculum in response to student interest in the topic. The module's focus is active, collaborative, and inquiry-based learning.	**Standard A: Teachers of science plan an inquiry-based science program for their students. In doing this, teachers** • develop a framework of yearlong and short-term goals for students. • select science content and adapt and design curriculum to meet the interests, knowledge, understanding, abilities, and experiences of students. • select teaching and assessment strategies that support the development of student understanding and nurture a community of science learners.
Student inquiry is encouraged by all activities in the module. The module promotes discourse among students, and challenges students to accept responsibility for their learning. The use of the 5E instructional model with collaborative learning is an effective way of responding to diversity in student backgrounds and learning styles.	**Standard B: Teachers of science guide and facilitate learning. In doing this, teachers** • focus and support inquiries while interacting with students. • orchestrate discourse among students about scientific ideas. • challenge students to accept and share responsibility for their own learning. • recognize and respond to student diversity and encourage all students to participate fully in science learning. • encourage and model the skills of scientific inquiry, as well as the curiosity, openness to new ideas and data, and skepticism that characterize science.
There are a variety of assessment components provided in module. Answers are provided to help teachers analyze student feedback.	**Standard C: Teachers of science engage in ongoing assessment of their teaching and of student learning. In doing this, teachers** • use multiple methods and systematically gather data about student understanding and ability. • analyze assessment data to guide teaching.
The answers provided for teachers model respect for the diverse ideas, skills, and experiences of all students. Students work collaboratively in teams to complete activities in the module. Discussion activities in this module model the rules of scientific discourse.	**Standard E: Teachers of science develop communities of science learners that reflect the intellectual rigor of scientific inquiry and the attitudes and social values conducive to science learning. In doing this, teachers** • display and demand respect for the diverse ideas, skills, and experiences of all students. • structure and facilitate ongoing formal and informal discussion based on a shared understanding of rules of scientific discourse. • model and emphasize the skills, attitudes, and values of scientific inquiry.

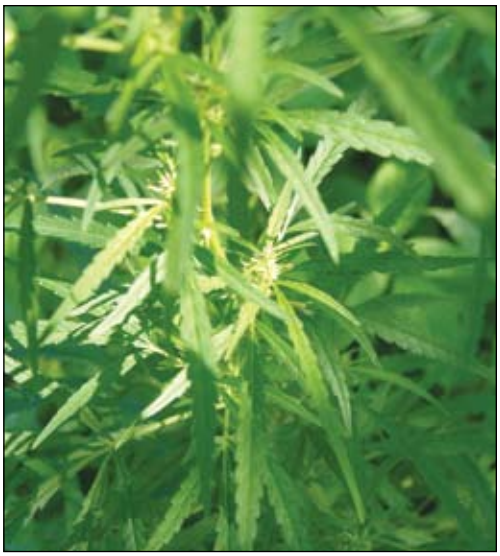
Get A Clue
Correlation to the National Science Education Standards

The Content Standards	
Get A Clue activity	
Pre-lab Activities **Wet-lab Activities** **Additional Activities**	**Standard A (Science as Inquiry) : As a result of activities in grades 9-12, all students should develop** **1. abilities necessary to do scientific inquiry.** • Identify questions and concepts that guide scientific investigations • Use technology and mathematics to improve investigations and communications • Formulate and revise scientific explanations and models using logic and evidence • Recognize and analyze alternative explanations and models • Communicate and defend a scientific argument **2. understanding about scientific inquiry.**
Introduction **Wet-lab Activities** **Pre-lab Activities**	**Standard C (Life Science): As a result of their activities in grades 9-12, all students should develop understanding of** **1. the cell.** • Cells store and use information to guide their functions • Cells can differentiate, and complex multicellular organisms are formed as a highly organized arrangement of differentiated cells **2. molecular basis of heredity.** • In all organisms, DNA carries the instructions for specifying organism characteristics • Changes in DNA occur spontaneously at low rates
Post-lab Activities	**3. biological evolution.** • Species evolve over time.
Wet-lab Activities **Post-lab Activities** **Additional Activities**	**Standard E (Science and Technology): As a result of activities in grades 9-12, all students should develop understanding of** **1. abilities of technological design.** **2. science and technology.** • Scientists in different disciplines ask questions, use different methods of investigation, and accept different types of evidence to support these explanations • Science often advances with the introduction of new technologies • Creativity, imagination, and good knowledge base are all required in the work of science and engineering • Science and technology are pursued for different purposes
Pre-lab Activities **Additional Activities**	**Standard F (Science in Personal and Social Perspectives): As a result of activities in grades 9-12, all students should develop understanding of** **1. personal and community health.** **5. human-induced hazards.** **6. science and technology in local, national, and global challenges.**
All	**Standard G (History and Nature of Science): As a result of activities in grades 9-12, all students should develop understanding of** **1. science as a human endeavor.** **2. nature of scientific knowledge.** **3. historical perspectives.**

INTRODUCTION

This module allows students to play the role of crime scene investigators, using DNA evidence to determine the culprit in a case. It fulfills the following learning objectives:

1. To identify the role of restriction enzymes in DNA restriction analysis.
2. To describe the technique of DNA gel electrophoresis.
3. To list the applications of DNA restriction analysis (DNA fingerprinting).
4. To explain why DNA fingerprinting can be used to solve crimes or to determine paternity.
5. To interpret gel electrophoresis results.



One of the uses of DNA fingerprinting is linking marijuana to different geographic areas.

BACKGROUND

DNA fingerprinting represents an incredibly useful and versatile tool for forensic scientists and geneticists who are seeking to name the father of a child, to diagnose an inherited illness, nab the perpetrator of a crime, or to solve some other mystery of identity. But because of the popularity of criminal detection shows on TV, many people most often think of DNA fingerprinting in connection with the guilt or innocence of suspects at a crime scene.

The inventor of DNA fingerprinting, British scientist Alec Jeffreys, first used the technique to settle a legal dispute in 1985. In order to confirm the identity of a boy who was trying to return from Ghana to the United Kingdom, Jeffreys compared the boy's DNA to that of his relatives. The child was allowed to return on the basis of the genetic evidence presented by Jeffreys. The next year, Jeffreys successfully identified the culprit in two murders committed in England. These crimes – known as the Footpath Murders – were the first to be solved by DNA fingerprinting.

In a Winston-Salem, North Carolina, case, 20-year-old Darryl Hunt was convicted in 1985 of raping and murdering a woman. Many years later, DNA collected from Hunt and other suspects was compared to the victim's DNA. This process of DNA fingerprinting revealed another man to be the culprit. The culprit confessed to the crime, and Hunt was released from prison on Christmas Eve 2003.

DNA fingerprinting is being used to look again for clues to many old criminal cases that occurred before this scientific technique was available. You can read more about Hunt and similar cases at the website of The Innocence Project (http://www.innocenceproject.org). Located at the Cardozo Law School in New York City, the Innocence Project is well known for using DNA evidence to clear the names of people who are in prison for crimes they did not commit.

Some scientists work in the related field of wildlife forensics, in which they use DNA as evidence for crimes such as poaching or the importation of protected species. DNA fingerprinting has even been used to determine relationships among marijuana plants, in order to potentially link production of this drug to different geographic areas.

DNA studies can be used for solving mysteries of the historical past. In 1998, a sample of mitochondrial DNA was used to officially identify the formerly anonymous individual buried at the Tomb of the Unknown Soldier in Washington, DC. In the same year, an article in the scientific journal Nature analyzed

evidence from Y chromosomes to suggest the possibility that Thomas Jefferson, or one of his male relatives, fathered a child of Sally Hemings (1773-1835), a slave who lived at Jefferson's home, Monticello.

DNA fingerprinting has also been used by individuals to locate distant ancestors and relatives. In Spring 2005, the National Geographic Society and IBM began a five-year project to use DNA to reconstruct a genealogy of the world's populations.

Finally, and just as importantly, DNA fingerprinting has numerous uses in the field of medicine – to understand, prevent, and diagnose disease. The technique has been applied to discover relationships between different viral strains in the field of epidemiology; to diagnose sickle cell anemia; and to determine susceptibility to a variety of inherited conditions, including cystic fibrosis and familial Alzheimer's.

ALTERNATIVE DNA FINGERPRINTING SCENARIOS

From Bio-Rad's Forensic DNA Fingerprinting Kit Instruction Manual

DNA typing, DNA profiling, and DNA fingerprinting are all names for the same process, a process which uses DNA to show relatedness or identity of individual humans, plants, or animals. DNA typing has become the subject of much debate and interest because of its uses for forensics analysis in prominent criminal cases such as the O. J. Simpson case. The applications of DNA typing, however, are much broader than forensic science alone and are having a profound impact on our society.

DNA typing is used in forensics, anthropology, and conservation biology not only to determine the identity of individuals but also to determine relatedness. This process has been used to free innocent suspects, reunite children with their relatives, identify stolen animals, and prove that whale meat has been substituted for fish in sushi. It is used in times of war to help identify the remains of soldiers killed in combat. It is also being used to find genetic linkages to inherited diseases. In addition, scientists are learning a great deal about our evolutionary history from DNA analysis.

Each of the following paragraphs describes a scenario in which DNA has been used to show how individuals are related to each other, or to show that a person is (or is not) the perpetrator of a crime. These scenarios provide a context for using DNA typing for use in teaching molecular biology, conservation biology, and biotechnology. Have your students research a scenario that is interesting to them and present their findings to the class.

DNA typing has been used to identify sushi that contain meat from endangered species, such as whales and dolphins.

1. Food identification (endangered species identification).

The purity (or impurity) of ground beef has been proven using DNA typing. Hamburger has been shown to often be a mixture of pork and other non-beef meats. Using portable testing equipment, authorities have used DNA typing to determine that the fish served in sushi was really meat from whales and dolphins. These are, many times, endangered species that are protected by international law.

2. Accused and convicted felons set free because of DNA typing.

A man imprisoned for 10 years was released when DNA testing, unavailable when he was convicted, was used to show that he could not have been the rapist. Statistics show that about 1/3 of all sexual assault suspects are freed as a result of DNA testing.

3. Identifying human remains.

Scientists have used DNA typing to confirm that the body in the grave was (or was not) the person that was supposed to be there. Bones found in Russia are believed to be those of the Romanovs, Russia's last imperial family. Czar Nicholas II and his family were executed by the Bolsheviks in 1918. Experts from around the world have been studying the bones to match skulls, teeth, and other features with photographs. DNA from the bones will be compared to that of known descendants to determine whether the bones do indeed belong to the czar and his family.

4. Determining relatedness of humans.

DNA typing has shown that the 5,000 year old "Iceman" found in a melting glacier is most closely related to modern Europeans. ("Iceman Gets Real." Science, vol. 264:1669. June 17, 1994.) The DNA typing evidence also "removes all the suspicions that the body was a fraud—that it had been placed on the ice," says Svante Paabo of the University of Munich. (Science, vol. 264:1775. June 17, 1994).

5. Studying relatedness among ancient peoples.

DNA found at archeological sites in western Montana is being used to help determine how many related groups of people (families) lived at a particular site. (Morell, Virginia. "Pulling Hair from the Ground." Science, vol. 265:741-745 August 1994.)

6. DNA testing of families.

DNA testing of families has been used in Argentina and El Salvador to identify the children of at least 9,000 citizens of these countries who disappeared between 1975 and 1983, abducted by special units of the ruling military and police. Many of the children born to the disappeared adults were kidnapped and adopted by military "parents" who claimed to be their biological parents. After genetic testing of the extended family revealed the true identity of a child, the child was placed in the home of its biological relatives. It was feared that transferring a child from its military "parents" who were kidnappers, but who had reared the child for years, would be agonizing. In practice, the transferred children became integrated into their biological families with minimal trauma.

7. Identifying organisms that cause disease.

Eva Harris, a UCSF scientist, is helping scientists in Nicaragua and Ecuador to learn to use DNA technology to detect tuberculosis, and identify the dengue virus and various strains of Leishmania. Other available tests cause waits of many weeks while disease organisms are cultured and sent to foreign labs to be identified. (Marcia Barinaga, "A Personal Technology Transfer Effort in DNA Diagnostics." Science, vol. 266:1317–1318. Nov. 25, 1994.)

8. Identifying birth parents (paternity testing).

Girls in Florida were discovered to have been switched at birth when one girl died of a hereditary disease. The disease was not in her family, but was known to be in the family of another girl, born in the same hospital and about the same time she was born.

9. Proving paternity.

A woman, raped by her employer on Jan. 7, 1943, her 18th birthday, became pregnant. The child knew who her father was, but as long as he lived, he refused to admit being her father. After the man died, DNA testing proved that she was his daughter and she was granted a half of his estate. ("A Child of Rape Wins Award from Estate of Her Father." New York Times, July 10, 1994.)

10. Determining effectiveness of bone marrow transplants.

"DNA fingerprinting can help doctors to monitor bone marrow transplants. Leukemia is a cancer of the bone marrow and the diseased marrow must be removed. The bone marrow makes new blood cells, so the leukemia sufferer will die without a transplant of healthy marrow. Doctors can quickly tell whether the transplant has succeeded by DNA

typing of the patient and the donor. If the transplant has worked, a fingerprint from the patient's blood shows the donor's bands. But if the cancerous bone marrow has not been properly destroyed, then the cancerous cells multiply rapidly and the patient's own bands predominate." ("Our Ultimate Identity Card in Sickness and in Health," in "Inside Science", New Scientist, Nov. 16, 1991.)

11. Proving relatedness of immigrants.
DNA fingerprinting has been used as proof of paternity for immigration purposes. In 1986, Britain's Home Office received 12,000 immigration applications from the wives and children of Bangladeshi and Pakistani men residing in the United Kingdom. The burden of proof is on the applicant, but establishing the family identity can be difficult because of sketchy documentary evidence. Blood tests can also be inconclusive, but DNA fingerprinting results are accepted as proof of paternity by the Home Office. (DNA fingerprints, source unknown: Based on A. J. Jeffreys et al., "Positive Identification of an Immigration Test-Case Using Human DNA Fingerprints." Nature, vol. 317:818–819, 1985.)

12. Confirming relatedness among animals.
Scientists who extracted DNA from the hair of chimpanzees throughout Africa now have evidence that there might be a third species of chimpanzee. At the same time they have learned things about chimp behavior and kinship patterns that would have once taken years to theorize. They discovered a group of chimps living in western Africa to be genetically distinct from the chimps living in other parts of Africa, suggesting that the group may be an endangered species. They have discovered that male chimps living in a given area are often as closely related as half-brothers, and many so-called sub-species may all be part of a single species. The male chimps' relatedness may explain why, unlike other primates, the males are quite friendly to each other.

Scientists used DNA to investigate the possibility of the existence of a third species of chimpanzee.

13. DNA testing of plant material puts murderer at the scene.
Two small seed pods caught in the bed of his pick-up truck put an accused murderer at the murder scene. Genetic testing showed that DNA in the seed pod exactly matched the DNA of a plant found at the scene of the murder. The accused had admitted he had given the victim a ride, but he denied ever having been near the crime scene.

THE PROCESS OF DNA FINGERPRINTING

The process of DNA fingerprinting involves several key steps. First, DNA is obtained from a small sample of body fluid or tissue such as blood, saliva, or hair. Next, scientists amplify (increase) the amount of DNA by using a technique known as PCR (polymerase chain reaction). Restriction enzymes are then used to cut the DNA into fragments that are unique to each individual. Finally, gel electrophoresis sorts these fragments according to size.

RESTRICTION ENZYMES

Also known as **DNA restriction analysis**, the technique of DNA fingerprinting requires the knowledge of many molecular genetic concepts — how **restriction enzymes** function, for example, and the mechanism underlying **gel electrophoresis**.

Acting as tiny, molecular scissors, restriction enzymes cut DNA at very specific sites to create smaller fragments of DNA. These enzymes scan the genetic code of guanines, cytosines, adenines, and thymines (Gs, Cs, As, and Ts) until they find a particular sequence of nucleotides, at which point they make cuts in the DNA.

Typically, restriction enzymes identify palindromic sequences in the DNA strands in which they make cuts. These genetic palindromes are similar to verbal palindromes, because they say the same thing when read both backwards and forwards. The words "mom" and "dad" are examples of verbal palindromes. The phrase "never odd or even" also reads the same backwards and forwards.

A palindromic sequence in DNA is one where the 5' to 3' base pair sequence is identical on each strand (the 5' and 3' ends referring to the chemical structure of the DNA). Each of the double strands of DNA is complementary to the other: adenine pairs with thymine, and guanine with cytosine.

ENZYMES AND PALINDROMES

Restriction enzymes (also known as restriction endonucleases) recognize and cut within specific palindromic sequences, known as **restriction sites**, in the genetic code. An example of a restriction enzyme, HaeIII, searches the genetic code until it finds this particular stretch of four nitrogen bases:

GGCC
CCGG

Once HaeIII finds the GGCC sequence, it **cleaves** (cuts) the DNA between the GG and CC bases. HaeIII would look for the GGCC sequence shown in bold letters in this portion of DNA:

TGACGTTCGA**GGCC**AG
ACTGCAAGCT**CCGG**TC

And it would cut the DNA into two pieces, right down the middle of the GGCC sequence:

TGACGTTCGA**GG** **CC**AG
ACTGCAAGCT**CC** **GG**TC

These straight cuts produce what are known as **blunt ends**.

NAMES

The names of restriction enzymes are related to the types of bacteria in which the enzymes are found, as well as to the order in which they are identified and isolated. EcoRI, for example, comes from the R strain of the *E. coli* bacteria. EcoRI was the first restriction enzymes discovered in *E. coli*, so "I" is used in its name.

Contrary to HaeIII, which makes clean cuts to produce blunt ends, other restriction enzymes make staggered cuts. (Think of a zigzag pattern.) EcoRI, for instance, searches for the following palindromic sequence:

GAATTC
CTTAAG

Rather than make a blunt cut, EcoRI cuts between the G and A on both the top and bottom strands. The G and A are highlighted in bold in both strands shown below:

GAATTC
CTTA**AG**

EcoRI cuts in different places on the top and bottom strands:

G **A**ATTC
CTTA**A** **G**

Because the enzyme produces a staggered cut, the ends of the resulting DNA fragments are referred to as overhanging or **sticky ends**. Since fragments with complementary sticky ends can be combined in different ways to create new molecules, sticky ends are useful in the field of recombinant DNA technology.

GEL ELECTROPHORESIS

Restriction enzymes cut DNA to produce fragments of different lengths. In order to sort these fragments according to their sizes, scientists use a technique known as **gel electrophoresis**. The gel through which the DNA moves is made of **agarose**, a seaweed derivative. Agarose is added in powder form to a **buffer** that balances the pH.

After it is poured into a casting tray, the agarose solidifies into a semi-solid gel. A plastic structure known as a comb is inserted into the solidifying agarose. Removal of this comb creates small rectangular wells into which the DNA samples are deposited.

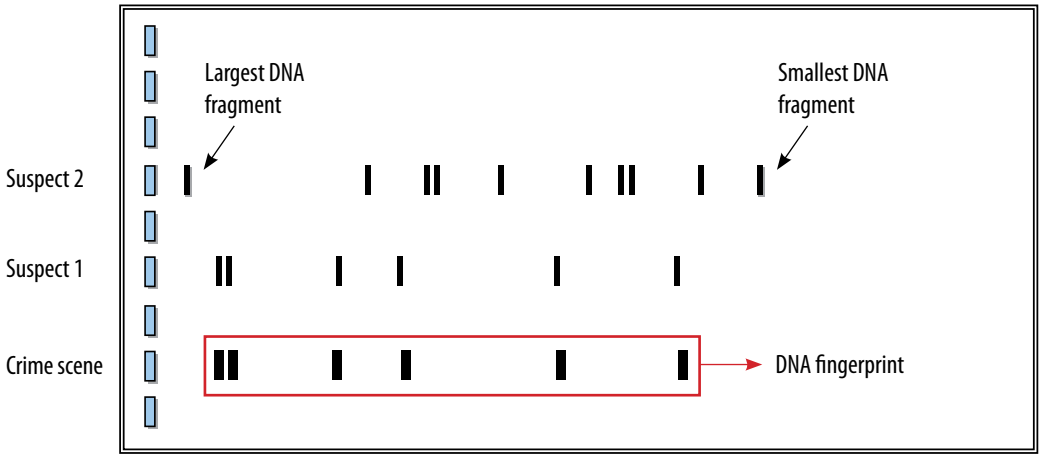
As the name "electrophoresis" implies, an electric current drives the DNA from one end of the gel to the other. DNA's negative phosphate groups play an important role in the process. In this case, the negatively charged DNA fragments move toward the positive end of the gel.

Electrophoresis works on the premise that different-sized DNA fragments move at different rates through a gel matrix. Long DNA fragments with many base pairs have more bulk and thus move slowly in comparison to small, faster pieces of DNA (Figure 1).

After a DNA sample finishes moving through the gel, a distinct banding pattern shows where the different DNA fragments have ended up. In this way, the fragments are sorted according to their sizes. The banding pattern that results from this is a unique DNA fingerprint that, like the fingerprints on one's hand, differs from one person to another. (The exceptions are identical siblings, such as twins or triplets, who share the same genetic fingerprints.)

This ability to compare DNA fingerprints enables us to determine the identity of the individual to whom, for instance, a piece of evidence at a crime scene may belong.

FIGURE 1: GEL ELECTROPHORESIS PRODUCES A DNA FINGERPRINT



RESTRICTION FRAGMENT LENGTH POLYMORPHISMS

Although 99.9% of each person's DNA is the same as that of other humans, DNA fingerprinting is possible because each person—with the exception of identical twins—has a unique restriction fragment length polymorphism (RFLP), This RFLP is the person's genetic "fingerprint."

The .1% difference in DNA can be attributed to differences in sequences of genetic code. Such differences often result from one of two causes:

1) repeat sequences

2) mutations within a genetic sequence.

Restriction sites on one person's DNA may be located in different regions from the restriction sites on another person's DNA. Restriction enzymes will thus cut the DNA of two different individuals in different places—producing "polymorphisms," or variations in fragment length.

Because these different-sized fragments create different banding patterns in a gel, scientists often use RFLP analysis (DNA fingerprinting) when investigating forensic evidence for a crime scene, or for determining the paternity of a child.

RFLPs are also used to identify genes associated with certain diseases. Huntington's disease, for example, is characterized by repeats in three nucleotides, CAG. Differences in banding patterns are used to determine genes potentially associated with the disease.

VARIABLE NUMBER TANDEM REPEATS

One of the main ways in which one person's DNA differs from that of another is in the number of VNTRs it contains. A variable number tandem repeat (VNTR) is a pattern of base pairs that occurs several times in a length of DNA. Thus, when restriction enzymes cut DNA, the resulting fragment sizes will differ depending on the number of repeats present between restriction sites.

Identical twins are the only humans whose DNA fingerprints are the same.

This number of repeats varies from person to person.

This DNA sequence is being cut by HAEIII:

GGCCTGACGTTCGA**GGCC**AG
CCGGACTGCAAGCT**CCGG**TC

HAEIII cuts between GG and CC, and three segments result:

GG|**CC**TGACGTTCGA**GG**|**CC**AG
CC|**GG**ACTGCAAGCT**CC**|**GG**TC

However, when a repeat sequence, such as CAG, exists in the DNA fragment, the length of the middle segment changes from 14 to 20 base pairs (in bold):

GG|**CC**TGA**CAGCAG**CGTTCGA**GG**|**CC**AG
CC|**GG**ACT**GTCGTC**GCAAGCT**CC**|**GG**TC

MUTATIONS

Although more rare than VNTRs, mutations can also lead to changes in base sequence, which in turns lead to the elimination of restriction sites. For example, a sequence GAATTC is normally recognized by a restriction enzyme:

GAATTC → **G** **A**ATTC
CTTAAG CTTAA G

But a mutation of this sequence to the sequence below results in no recognition by the restriction enzyme:

GTATTC → ***No Cut***
CATAAG

If one person has a normal GAATTC sequence, and another person has a mutation, each will produce different-sized fragments of DNA.

THE USE OF PROBES IN ANALYSIS OF VNTR'S

Probes are known sequences of DNA that will bind to their complementary DNA sequences. A probe with the sequence 5'–TATATAAGCTC–3' will bind to a DNA fragment on a membrane with the sequence 3'–ATATATTCGAG–5'.

DNA probes, which are tagged in a way that allows them to be seen, are used to make the differences among DNA fragments in an electrophoresis gel visible. They create the pattern of bands that is known as a DNA fingerprint.

The use of probes provides a very accurate way to compare samples of DNA. If two different, appropriate probes are used, the chance of two unrelated people having the same pattern of bands is about one in 30 billion. Given that the current world population is about 6.5 billion, this is a very highly reliable method of identification.

DNA fingerprinting is powerful because of the combined analysis of a number of VNTR loci (locations) on different chromosomes. The term VNTR refers to the variable sequence rather than the method used to detect it. Several PCR-based methods can be used to detect VNTRs.

RFLP is used to detect the variability in the number of repeats. The number of repeats at a single VNTR locus cannot distinguish an individual from the rest of the population; the combined results from a number of loci produce a pattern unique to that person. There are thousands of RFLP loci or DNA segments that can be used in humans.

Some populations have less variation in a particular DNA segment than do other populations. The amount of variation has an effect on the statistical odds of one individual having the same sequence.

CODIS (**CO**mbined **D**NA **I**ndex **S**ystem) is a federally maintained database used by law enforcement officials. The FBI incorporates an average of 13 sites into its profiles. In studying 26 different bands it would be almost impossible to find two unrelated individuals with the same DNA profile. The odds of a match in this case are well more than one in a hundred billion.

PRE-LAB

The teacher selects from one of the provided scenarios ("The Tempting Tiara" or "The Irresistible iPods"). Students role play the selected crime scene scenario.

A section of the classroom is dressed up as the scene of the crime. Yellow "CRIME SCENE INVESTIGATION – DO NOT CROSS" tape is in place. Some classmates will act as detectives collecting various clues. The physical evidence includes one fingerprint, several drops of blood, and some fibers apparently left by the thief.

This activity teaches students how to sort the physical evidence and to understand the basics of DNA fingerprinting. They will sort DNA fragments by size, and compare the RFLPs of each suspect with those of the crime scene DNA. These skills prepare the students for the wet-lab activity.

WET-LAB

After a role-play in which students pretend to be DNA fragments moving through the gel during the electrophoresis process, students are ready to learn how to perform restriction analysis using gel electrophoresis.

In the wet-lab activity, students are told that

DNA isolated from a drop of "blood" left at the crime scene (specifically from white blood cells in the blood) was amplified using the polymerase chain reaction (PCR). Using DNA restriction analysis in the wet lab to compare the DNA of this blood sample to DNA from each suspect, students can determine which suspect is guilty and which suspects are innocent.

POST-LAB/ ADDITIONAL ACTIVITIES

Additional activities for this module include the following components and resources:

- DNA Restriction Enzyme and Probe Activity
- Taking Fingerprints Activity
- Blood Typing: Practice Using Punnett Squares
- The Ima Mystery Case
- A Paternity Case
- A Crime on Campus
- Get a Clue Quiz Game

Correlation with Restriction Digest

This post-lab activity offers students an opportunity to integrate math and science. Students analyze electrophoresis results from the wet lab and perform standard-curve analysis to identify DNA fragments of unknown length for suspect 2. By plotting the distances that DNA fragments travel on the gel against the logarithm of base-pair length for each fragment from the crime scene DNA, students produce a standard curve. They then use this standard curve to predict the base-pair length of fragments for suspect 2. This activity is required for AP Biology.

Other additional activities illustrate the practicality of using DNA evidence in real-life situations, either to establish paternity or to determine the innocence or guilt of a suspect in a crime. The Quiz Game allows students to quickly review content within the module in a fun, interactive game show format.

MOCK TRIAL

By conducting a mock trial, students may integrate this module with English or social studies. Students may create their own scenario for trial, or reenact a famous historical or literary trial or investigation. Two examples are provided in the "Interdisciplinary Bridges" section of the Get a Clue notebook. One involves the trial of Lizzie Borden; another uses the Russian novel The Brothers Karamazov. Additional examples of literary works that have crime scenes, paternity disputes, and other plot lines that can generate role-play in a mock trial are also provided at the end of the Get a Clue notebook.

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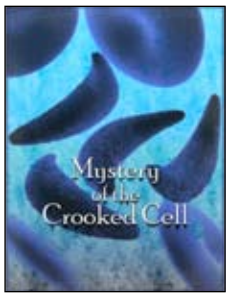
CONNECTION TO OTHER MODULES

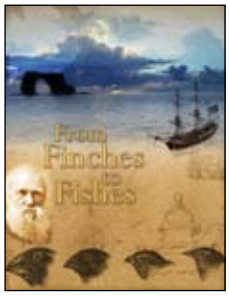
This module focuses on human forensic testing. The actual crime scene scenario selected is determined by the teacher. The objective of the wet lab is to enable students to understand some of the basic scientific principles involving DNA fingerprinting. This module along with the two additional wet-lab activities mentioned below provides students with first-hand experience of common techniques used by molecular biologists. Molecular biology examines nucleotide and amino acid sequences of DNA and proteins from different species.

MYSTERY OF THE CROOKED CELL



To enable students to build on content learned in Get a Clue, we strongly recommend Mystery of the Crooked Cell, a module developed by Boston University School of Medicine's CityLab. In Mystery of the Crooked Cell, students examine the genetic basis for sickle cell anemia. Students observe functional differences in the normal hemoglobin and sickle cell hemoglobin which result from a point mutation that changes the DNA. In both Get a Clue and Mystery of the Crooked Cell, students perform an electrophoresis experiment as a tool to obtain specific data.

FROM FISHES TO FINCHES



From Finches To Fishes provides an opportunity for students to increase their knowledge of molecular biology and refine their techniques of electrophoresis by separating proteins using precast polyacrylimide gels and vertical gel chambers.

SEQUENCE OF MODULES

A sequence relating the three modules is summarized below:

1. GET A CLUE

Students learn the structure and function of DNA. Students learn basic techniques of DNA gel electrophoresis.

2. MYSTERY OF THE CROOKED CELL

Students learn how changes in DNA structure lead to changes in protein structure. Students learn basic techniques of protein electrophoresis

3. FROM FINCHES TO FISHES

Students compare protein from the muscle cells of fish to determine which fish are most closely related. Since DNA determines protein, those fish which have the most similar proteins would be expected to have similar DNA and similar origins. This is a more advanced type of electrophoresis lab using polyacrylimide gels vertical gels to separate proteins.

GET A CLUE IMPLEMENTATION PLAN — PRE-LAB			
Activity	Estimated Time	Materials/Equipment	Purpose/Objectives/ Essential Question
Engagement	8 minutes	Crime Scene Scenario/ Props	**Purpose:** To discover and apply the concepts of restriction enzymes, electrophoresis, and DNA restriction analysis **Objectives:** • To identify a need for DNA restriction analysis • To develop through modeling the concept of DNA restriction analysis • To apply DNA restriction analysis to the identification of DNA fragments • To work cooperatively to analyze the results of the evidence analysis exercises **Essential Question:** What processes are necessary to solve the crime based on the evidence you have been given?
Exploration	30 minutes	Evidence Report Form for each group Copies of the crime scene, instructions Prepare five envelopes (8½ in. by 11 in.) as DNA evidence: labeled suspect 1, 2, 3, 4 and crime scene for each group of five students. 2-3 pairs of scissors for each group One roll of tape One envelope containing the blood evidence: Simulated Anti-A; B; and Rh Serum Blood typing tray; 3 stirring sticks Blood from suspect Instruction Sheet One envelope containing the fingerprint evidence: Fingerprint from the crime scene Fingerprints from one of the suspects One envelope containing fiber evidence: Results of the burn test Microscopic results	
Explanation/Elaboration	20 minutes	Enzyme Worksheet The Power of Discrimination Sheet	
Evaluation	10 minutes	Key Terms handout	

Alignment with NC Competency Goals	
Biology	
Goal 1 Objectives 1.01, 1.02, 1.03, 1.05 Goal 2 Objectives 2.02, 2.03, 2.04	Goal 3 Objectives 3.01, 3.04 Goal 4 Objectives 4.04

GET A CLUE PRE-LAB ACTIVITIES: UTILIZING THE 5E INSTRUCTIONAL MODEL

ENGAGEMENT

Students will role play one of the crime scene scenarios selected by the teacher.

A section of the classroom is prepared as the scene of the crime. Yellow "CRIME SCENE INVESTIGATION – DO NOT CROSS" tape is in place. Some classmates will act as detectives collecting various clues. The physical evidence from each scenario includes one fingerprint, several drops of blood, and some fibers that have been left by the robbers.

EXPLORATION

Divide students into four groups, each of which will act as a forensics team. Provide a copy of the scenario selected. Explain to students that their job is to solve the crime based on the physical evidence found by the crime scene investigators. Instruct students to follow directions provided to solve the crime. The teacher should act as a facilitator guiding students to look at all the evidence as they work together to solve the crime.

- Each group of students will examine fingerprints from the four suspects and compare those with the one found by the crime scene investigators.
- Each group of students will examine fibers found at the crime scene and determine their type.
- Each group should examine the blood evidence found at the crime scene.
    - Students will analyze the blood from one of the four suspects with regard to blood type (ABO and Rh system) and place their findings in the designated place on the board.
    - Students will analyze the DNA evidence from the blood and model the process of gel electrophoresis.

EXPLANATION/ ELABORATION

Each group will complete their blood evidence and place the results on the board. (ABO and Rh)

Ask each group to place their DNA fragments on the board.

Challenge each group to either support or refute the explanations given for each type of evidence.

Then lead a class discussion around the following topics:

- DNA structure/models of DNA
- Milestones in forensic DNA analysis
- DNA fingerprints vs. actual fingerprints
- Applications of DNA fingerprinting
- Making a DNA fingerprint (PCR, restriction enzymes and gel electrophoresis)
- Variations in DNA (mutations & VNTR)
- Why is DNA testing sometimes controversial?
- Validity of blood and DNA evidence

EVALUATION

Write a paragraph that gives a possible scenario for what might have happened based on the evidence. Describe how the physical evidence supports your hypothesis. Use as many of the terms provided as possible.

ENGAGEMENT ACTIVITY

Students will role play one of the crime-scene scenarios selected by the teacher.

A section of the classroom is prepared as the scene of the crime. Yellow "CRIME SCENE INVESTIGATION – DO NOT CROSS" tape is in place. Some classmates will act as detectives collecting various clues. The physical evidence from each scenario includes one fingerprint, several drops of blood, and some fibers which have been left by the robbers.

At the end of the skit, a student playing the role of the FBI agent selects four students at random as the four suspects. The FBI agent lays down the subpoena and swabs each suspect's cheek for DNA evidence.

Optional equipment: Four cotton swabs in four small plastic bags; folders/sheets of paper marked "**SUBPOENA**".

ENGAGEMENT ACTIVITY SCENARIO 1: THE CASE OF THE TEMPTING TIARA

CHARACTERS NEEDED

Narrator, Sara Staggerdcut, Dr. Edmond Strater, Chief Detective X. Hammond, FBI agent

Narrator: The scene of the crime is Destiny High School. Chief Detective X. Hammond is already on the scene, interviewing students and teachers. The head of the Crime Scene Unit, Sara Staggerdcut is also on the scene, dusting for prints and searching for physical evidence.

Sara Staggerdcut: Looks like a "smash and grab" job, Chief. The culprit broke into this glass case and stole the dazzling homecoming tiara that was going to be awarded to the homecoming queen at halftime of this Friday's football game.

Narrator: Dr. Edmond Strater, the school's principal, comes onto the scene.

Dr. Edmond Strater: Detective X. Hammond, you have to find the homecoming tiara.

Detective X. Hammond: We'll do our best. Is there anything you can tell us that may give us a lead?

Dr. Edmond Strater: Well, the tiara has traditionally been displayed in the foyer of the main hall the week prior to homecoming. Its beauty is magnetic and it has enticed girls from a young age to dream of one day wearing it. For generations, it's served to inspire school spirit. That school spirit has carried us to 12 straight homecoming victories. We have to get it back before Friday's game.

Detective X. Hammond: When do you think this happened?

Dr. Edmond Strater: It happened during the pep rally. All of the students and staff were in the gym, and it was loud enough that nobody would have heard the glass being broken. It must have been a pretty easy getaway. We've got to get it back before Friday's game.

Detective X. Hammond: We will do everything we can. Sara, how's the Crime Scene Unit doing?

Sara Staggerdcut: Well, Chief, we haven't found anything yet, but we're dusting for fingerprints right now. I also think that, with all of this broken glass, there could be a chance that the thief cut themselves. We'll have to spray some luminol to search for blood.

Detective X. Hammond: Great, collect whatever evidence is here and take it back to the lab. I'll stick around here and find out who would have the means and the motive to pull this off. We can meet up tomorrow to share information.

- -

Narrator: Sara Staggerdcut and her forensics team package and label all evidence found and take it back to the forensics lab. Detective X. Hammond leaves to finish his interviews. The next day, Detective X. Hammond and Sara Staggerdcut meet in the forensics lab.

Sara Staggerdcut: I've got great news for you, Chief. The perp was pretty sloppy. The Crime Scene Unit was able to find three pieces of evidence.

Detective X. Hammond: Great, what were they?

Sara Staggerdcut: First is a fingerprint that we found on the silver stand that held the tiara. Dr. Edmond Strater said that the silver was polished daily to remove any fingerprints. It sounds like he was pretty particular about it. So there's a good chance that the fingerprint belongs to the criminal.

Detective X. Hammond: That is good news. What else you got?

Sara Staggerdcut: We sprayed down some of the shards of glass with luminol and we found some blood. We collected a sample and brought it back here to the lab, but we still need to run some tests on it.

Detective X. Hammond: You said there were three pieces of evidence.

Sara Staggerdcut: We also found some fibers at the crime scene. It looks like, as the perp was grabbing the tiara, they got their shirt or other clothing stuck. When they ran off with the tiara, a small piece of clothing was torn off. We're not sure yet what the fiber is made from, but we'll be testing it today. But all of this evidence may be no good without suspects to compare the fiber, fingerprint, and DNA evidence to.

Detective X. Hammond: Well, I've got good news for you, we have four suspects.

First is **Mia Goddess**, last year's homecoming queen. The rumor around school was that she stole 200 ballots last year and voted for herself on every one of them, though nothing could ever be proven. She's very stubborn, and has been heard saying numerous times that she didn't want to give up her crown.

Our second suspect is **Alfred Crank**, the rich father of Heather Crank, a girl who was eliminated from the competition last week. We know Heather didn't do it because the entire school saw her try to trip one of the leading contenders for queen in the pep rally. When Heather was eliminated last week, Alfred complained to Dr. Edmond Strater shouting, "Do you know who I am? You can't do this to my daughter, I promise you will pay for this!"

Suspect number three is **Brittany Show**. Brittany is an attention-seeking girl who is secretly attempting to catch the eye of the quarterback, on whom she has a crush.

Our last suspect is **Jacques Straup**, starting quarterback for Central High School, the school that Destiny High plays this week in the homecoming game. He may be trying to create a diversion that could give his team the edge. Though he's never been caught, he brags that last year he kidnapped mascots from three other high schools. Apparently he got the North High ram, the West High hedgehog, and the South High concrete donkey. Maybe this time he wanted something a little more challenging.

We were able to bring all four in for questioning. Of course, they all denied any involvement. Every suspect did, however, unknowingly leave fingerprints on the table in the interrogation room. Some of the fingerprints were smudged, but they may still be useful. A couple of the suspects have also donated blood in the past. Although none of their blood was still available for DNA testing, the blood bank did have records indicating the suspects' blood type. If we are able to match fingerprints, fibers, or blood type to any of our suspects, a judge may grant us a warrant to get DNA samples.

Sara Staggerdcut: Great! We should have enough evidence to narrow down our suspect pool. Let's get into the lab and get to work.

- -

The FBI agent now selects four students at random as suspects Mia Goddess, Alfred Crank, Brittany Show, and Jacques Straup. The FBI agent lays down the subpoena and swabs each suspect's cheek for DNA evidence.

ENGAGEMENT ACTIVITY SCENARIO 2: THE CASE OF THE IRRESISTIBLE iPODS

CHARACTERS NEEDED

Narrator, Sara Staggerdcut, Dr. Edmond Strater, Chief Detective X. Hammond, FBI agent

Narrator: The scene of the crime is Destiny High School. Chief Detective X. Hammond is already on the scene interviewing victims. The head of the Crime Scene Unit, Sara Staggerdcut is also on the scene, dusting for prints and searching for physical evidence.

Sara Staggerdcut: Looks like a "smash and grab" job, Chief. The culprit used a crow bar to break into lockers and has made off with 10 iPods.

Narrator: Dr. Edmond Strater, the school's new principal, comes onto the scene.

Dr. Edmond Strater: Why would someone want to steal 10 iPods? If they were stealing to use them, they would have only stolen one. This person must be planning on selling them. We should check out eBay.

Detective X. Hammond: You're probably right. I'll have my team go online and find out. If we see anything suspicious, we can buy an iPod and check it for clues. Sara, have we found anything here?

Sara Staggerdcut: Looks like a pretty clean job. The perp must have been wearing gloves, because there are no suspicious fingerprints on the lockers. There may be one piece of evidence, though. It looks like there may be a drop of blood on one of the jagged edges of a destroyed locker. Maybe the thief cut themselves breaking into it. We'll have to spray luminol to see if there is any blood here.

Detective X. Hammond: Great, collect whatever evidence is here and take it back to the lab. When you get there, go online to find out if our culprit is trying to sell his new iPods. I'll stick around here and find out who would have the means and the motive to pull this off. We can meet up tomorrow to share information.

- -

Narrator: Sara and her forensics team package and label all evidence found and take it back to the forensics lab. Detective X. Hammond leaves to finish his interviews. The next day Detective X. Hammond and Sara Staggerdcut meet in the forensics lab.

Sara Staggerdcut: I've got great news for you, Chief. We found an eBay user from this area that put 10 iPods up for sale yesterday morning. We tried to find out who that user was, but it was a dead end. They set up the account and posted their sale items using a public library computer. The seller's Paypal account was linked to a p.o. box. Tracking the owner of the p.o. box was also a dead end. Our thief was really good at covering their tracks.

Detective X. Hammond: I thought you said you had good news.

Sara Staggerdcut: I do, I bought one of the iPods from that eBay seller and had it shipped by next-day mail. When it got here, we hit the jackpot. In the charging jack there were some lint fibers, probably from being in someone's pocket. Also, the screen had one very clear fingerprint. We ran it through our database and couldn't find a match, so our perp is a first-time offender.

Detective X. Hammond: What about any evidence at the crime scene?

Sara Staggerdcut: More good news. We sprayed the locker with luminol and found out that it was blood that we found at the crime scene. We need to figure out what the blood type is and run a DNA restriction analysis. But all of this evidence may be no good without suspects to compare the fiber, fingerprint, and DNA evidence to.

Detective X. Hammond: Well, I've got good news for you, we have four suspects.

First is **JJ Shady**, a basketball player from a rival high school who reportedly has a mean streak in him. In his game last week at Destiny High School, he had one of the worst shooting nights of his career and his team lost. He was quoted in the local paper as saying, "Their fans were heckling me, that's why I played so poorly. I'll get them back, you can be sure of that."

Our second suspect is **Heather Sweetpea**. Heather used to date one of the students who had his iPod stolen. That is, until he dumped her last week. He told us that when he broke up with Heather, she wasn't sad. She was angry. He also said that she was one of the few people who knew that he always kept his iPod in his locker.

Suspect number three is **Bill Pilfer**, a known thief here at Destiny High School. In the past he has been caught stealing lunches and small amounts of money. He has also been suspected of stealing a calculator and three backpacks, though nothing could ever be proven. When I approached him, he shouted out, "I didn't steal anything," before I could even ask him a single question.

Our last suspect is **Melinda Green**, affectionately known to Destiny High School staff as "Mean Melinda Green." She's the mother of a troublemaking sophomore named Skipper, and she has been a thorn in the side of numerous

administrators. She came to Dr. Edmond Strater last month claiming that someone had stolen Skipper's iPod. Dr. Strater found out from some students that Skipper was so angry from getting assigned detention, he threw his iPod across the hall, breaking it. Dr. Strater suspected that Skipper wanted his mother to buy him a new iPod, so he told her it was stolen. Of course, Green didn't believe Dr. Edmond Strater when he told her this theory and shouted, "If you don't care that people are going around stealing iPods, then maybe I need to steal one and give it back to Skipper!"

We were able to bring all four in for questioning. Of course, they all denied any involvement. Every suspect did, however, unknowingly leave fingerprints on the table in the interrogation room. Some of the fingerprints were smudged, but they may still be useful. A couple of the suspects have also donated blood in the past. Although none of their blood was still available for DNA testing, the blood bank did have records indicating the suspects' blood type. If we are able to match fingerprints, fibers, or blood type to any of our suspects, a judge may grant us a warrant to get DNA samples.

Sara Staggerdcut: Great! We should have enough evidence to narrow down our suspect pool. Let's get into the lab and get to work.

The FBI agent now selects four students at random as suspects JJ Shady, Heather Sweetpea, Bill Pilfer, and Melinda Green. The FBI agent lays down the subpoena and swabs each suspect's cheek for DNA evidence.

EXPLORATION ACTIVITY

Divide students into four groups, each of which will act a forensics team. Provide a copy of the scenario selected. Explain to students that their job is to solve the crime based on the physical evidence found by the crime scene investigators. Instruct students to follow the directions provided to solve the crime. The teacher should act as a facilitator, guiding students to look at all the evidence as they work cooperatively to solve the crime.

• Students are to examine fingerprints from the four suspects and compare those with the one found by the crime scene investigators.

• Students will examine fibers found at the crime scene and determine their type.

• Each group should examine the blood evidence found at the crime scene.

  ○ Each group of students will analyze the blood from one of the four suspects with regard to blood type (ABO and Rh system) and place their findings in the designated place on the board.

  ○ Each group of students will analyze the DNA evidence found in the blood, and they will model the process of gel electrophoresis.

NAME _____

THE EVIDENCE REPORT

Put a + in each block that matches with the evidence found at the crime scene.

	Blood evidence Blood type	Frequency of ABO Blood Type in the U.S.	Fiber Type	Fingerprints	Results of DNA Evidence
Evidence	A+	34%			
Suspect 1					
Suspect 2					
Suspect 3					
Suspect 4					

ADDITIONAL INFORMATION ON BLOOD TYPES

FREQUENCY OF ABO BLOOD TYPES AND RH FACTORS IN THE US

Blood Type	Frequency Percentage	Blood Type & Rh Factor	Frequency Percentage
A	42	A+ A-	34 8
B	10	B+ B-	8 2
AB	4	AB+ AB-	3 1
O	44	O+ O-	35 9

Neo Science Teachers Guide; ABO Blood Typing Using Neo/Blood #20-213

OVER

AFTER YOU COMPLETE THE FIBER-IDENTIFICATION ACTIVITY

Using the information from the fiber burn test and the photo of the fiber under the microscope, what fibers make up the materials found at the crime scene?

_____

Our crime lab was able to trace the material dyed in that color to a specific retailer who sells clothes in this area. Their sales manager informed us that the stores in this area have sold more than 2,500 garments made from that material dyed that color in the past year. Therefore, we have determined that the chances of any one particular person in this area owning one of those garments is relatively high, and this evidence is not useful in helping to identify one single suspect. If other evidence is available, we may be able to get a warrant to search our suspects' homes to look for the piece of clothing that our mystery cloth was torn from.

INSTRUCTIONS FOR FINGERPRINTS ACTIVITY

MATERIALS NEEDED

- Fingerprints Data Sheet
- Magnifying glass
- Metric ruler

PROCEDURE

Your group will closely examine all fingerprints provided, compare and contrast them using the magnifying glass and metric ruler, and attempt to match the only fingerprint found at the crime scene to one of the suspects being investigated for the crime.

NOTE: In order to eliminate a suspect, the following criteria must be met:

(1) There must be a complete set of fingerprints for the suspect.
(2) The set of fingerprints must be of good quality.

REMEMBER: You will be analyzing more evidence found by the forensic team. No single piece of evidence collected, charted, and analyzed on the "Evidence Report" will eliminate possible suspects for this crime.

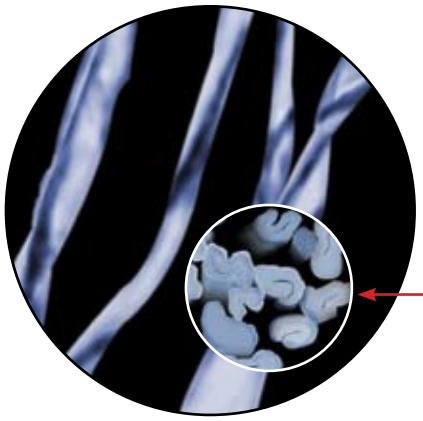
FIBER IDENTIFICATION

IDENTIFICATION USING A MICROSCOPE

One kind of evidence that may help solve our crime are the fibers that our forensics team gathered. Not much is known about the fibers other than that they came from cloth that was "Duke blue" in color. The forensics team needs to determine what the cloth was made from so that they can possibly identify its origin.

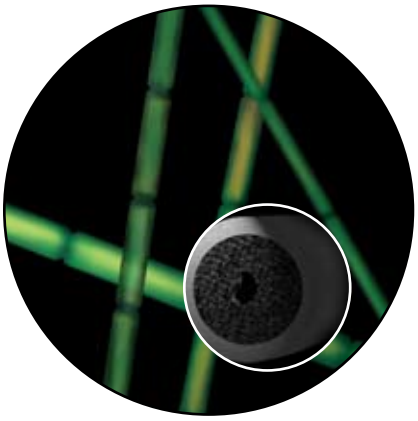
Our forensic scientists looked at the fiber under a microscope. They were able to look at the general shape of the fibers, as well as at the cross-section of the fiber. Below is a photo of our unknown fibers under the microscope, with a smaller photo of the cross-section of those fibers. The lab has also included a photo of other common fibers under the microscope. Compare our unknown fibers with the others to see if we can identify the evidence gathered by our forensics team.

What type of material do you think was found by the forensics team?



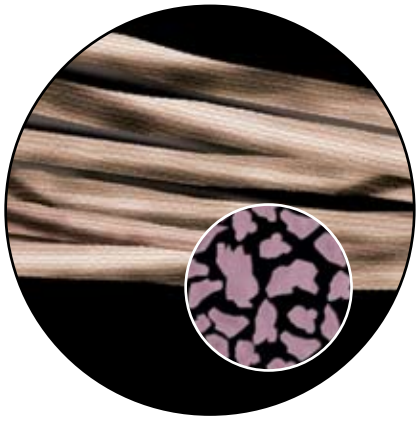
MYSTERY FIBERS UNDER THE MICROSCOPE

Cross section



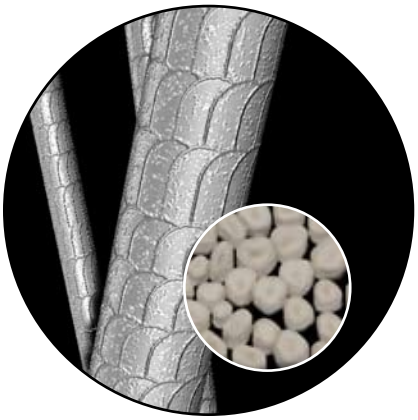
LINEN

Linen is a natural fiber that comes from the stem of a plant called flax.



SILK

Silk comes from the cocoons of silkworms.



WOOL

Wool is a natural fiber that comes from the hair of sheep or other similar mammals.

COTTON

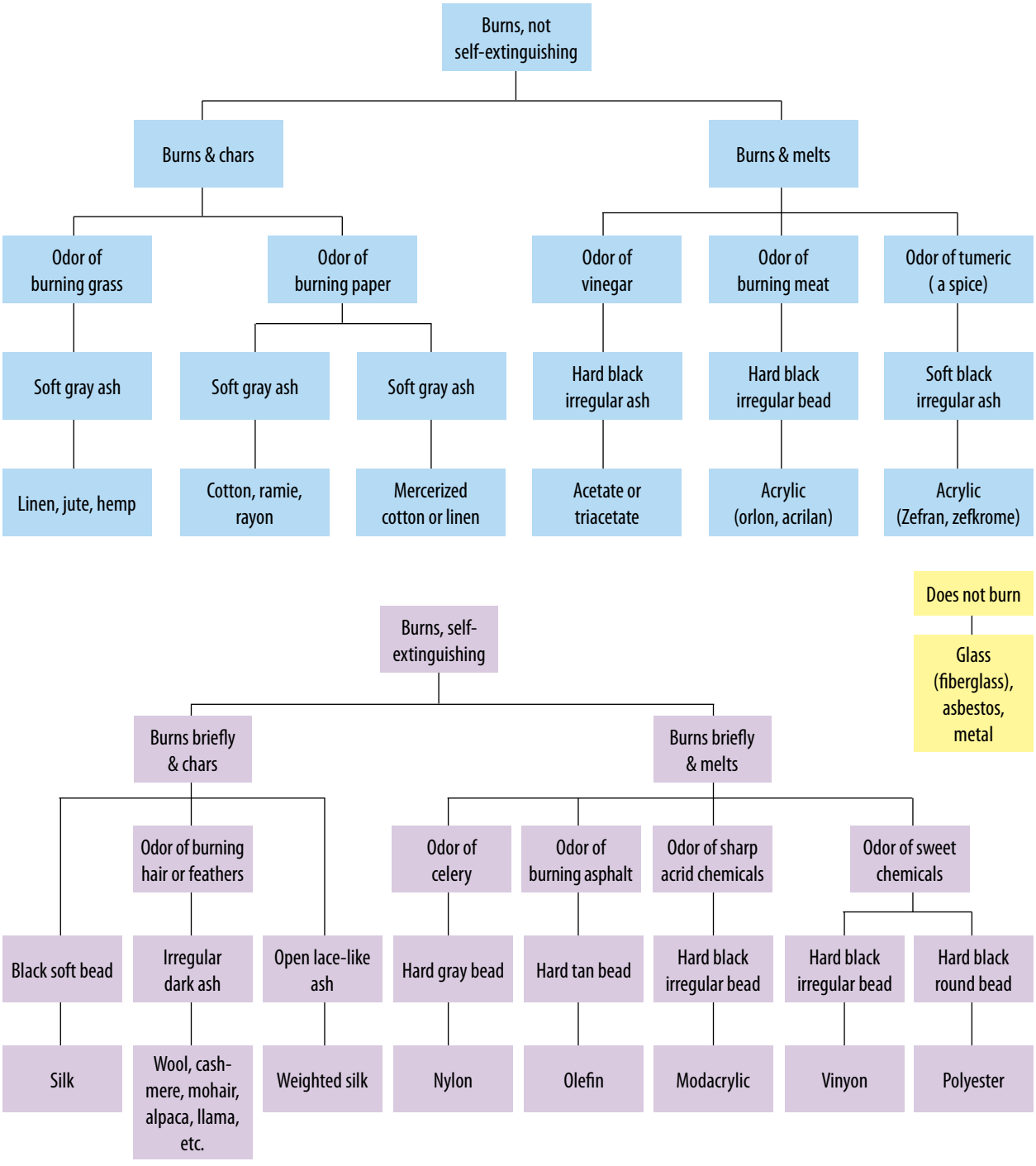
Cotton is derived from the cotton plant.

IDENTIFICATION USING THE BURN TEST

Forensic scientists will also subject the unknown fibers to a "burn test," during which the fiber is burned to determine its properties. To identify the fibers found at the crime scene, first read the description of the behavior of the unknown fibers when subjected to the burn test. Then use the chart below to identify the type of fiber found at the crime scene.

RESULTS OF THE BURN TEST

When subjected to the burn test, the unknown fibers burned and were not self-extinguishing. The fibers charred and smelled like burning paper. After the fiber had completely burned, it produced a soft, gray ash.



FINGERPRINTS

OBJECTIVES

Students will use their knowledge of crime scene evidence collection, as well as of fingerprinting techniques, to evaluate and analyze fingerprint evidence for this particular criminal case.

BACKGROUND INFORMATION

Pads on the human finger create patterns of ridges that leave imprints on surfaces touched. These imprints are referred to as fingerprints. It is believed that these "dermal" ridges provide the traction humans need for grasping objects. Fingerprints are useful in crime scene investigations because they are normally unique to each individual person, meaning that no two fingers will have exactly the same ridge characteristics. These characteristics do not alter with growth or age.

Fingerprints are produced when visible or invisible residue is left on surfaces that a person touches with his/her fingers. This residue can be collected for evaluation and analysis. With visible fingerprints, finely ground chalk

or coal powders are used. With invisible fingerprints (normally referred to as "latent fingerprints"), special chemical techniques (cyanoacrylate fuming and ninhydrin spray) can be used to make them visible.

Fingerprints can also be classified into three main patterns: arch, loop, and whorl. These patterns help distinguish fingerprints even more precisely.

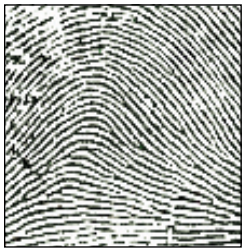
TYPICAL FINGERPRINT CLASSIFICATION

LOOP

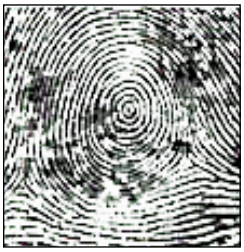
DOUBLE LOOP

CENTRAL POCKET LOOP

TENTED ARCH



PLAIN ARCH

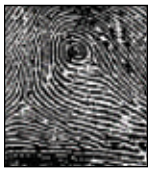


PLAIN WHORL

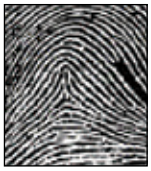
ACCIDENTAL

FINGERPRINT CLASSIFICATION IMAGES COURTESY OF http://www.fbi.gov/kids/k5th/whatwedo3.html

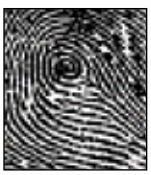
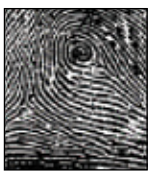
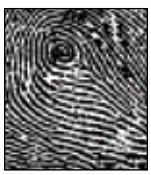
FINGERPRINTS DATA SHEET

FINGERPRINT EVIDENCE				
				
Unknown digit				

SUSPECT 1				
				
Left little	Left ring	Left middle	Left index	Left thumb

SUSPECT 2				
				
Right thumb	Right index	Right middle	Right ring	Right little

SUSPECT 3				
				
Right thumb	Right index	Right middle	Right ring	Right little

SUSPECT 4				
				
Right thumb	Right index	Right middle	Right ring	Right little

DETERMINING THE ABO-RH BLOOD TYPES OF SIMULATED BLOOD SAMPLES

Blood cells have surface proteins (called antigens) that determine an individual's blood type. In 1930, Dr.Karl Landsteiner, an Austrian physician, received the Nobel Prize in physiology for this important discovery.

In the ABO System, the type of antigen present on the surface of one's blood cells determines the blood type.

A person with Antigen A has type A blood
A person with Antigen B has type B blood
A person with both antigens A and B have type AB blood
A person with neither antigen has type O blood

Antibodies are blood plasma proteins.

A person with A surface antigens has anti-B antibodies.
A person with B surface antigens has anti-A antibodies.
A person with both A & B surface antigens produces no antibodies.
A person with no surface antigens has both anti-A and anti-B antibodies.

Blood typing in the ABO system is performed by using "antiserum" blood that contains specific antibodies.

Anti-A Serum contains anti-A antibodies.
Anti-B Serum contains anti-B antibodies.

To perform the blood-typing test, anti-A is mixed with a drop of blood and observed for agglutination (clumping). The same procedure is carried out with anti-B serum, which is also mixed with a blooddrop and observed for agglutination.

AGGLUTINATION REACTIONS IN THE ABO SYSTEM

ABO AGGLUTINATION REACTIONS		
Anti-A Serum	**Anti-B Serum**	**Blood Type**
Agglutination	No agglutination	A
No agglutination	Agglutination	B
Agglutination	Agglutination	AB
No agglutination	No agglutination	O

After the ABO blood type is determined, another important antigen on the surface of red blood cells called the Rh protein can be determined. This protein's name comes from the rhesus monkey in which it was first studied.

People who have this protein are "Rh-positive," and those who lack it are "Rh-negative."

- Rh-negative individuals who have been transfused with Rh-positive blood can produce Rh antibodies.
- If they are transfused again with a Rh-positive blood, they may develop a transfusion reaction, during which agglutination may occur.

Rh AGGLUTINATION REACTION

Rh AGGLUTINATION FACTOR	Rh FACTOR
Agglutination	+
No agglutination	–

THE BLOOD EVIDENCE: DETERMINATION OF BLOOD TYPE OF SUSPECT

AGGLUTINATION REACTIONS IN THE ABO SYSTEM

ABO AGGLUTINATION REACTIONS		
Anti-A Serum	**Anti-B Serum**	**Blood Type**
Agglutination	No agglutination	A
No Agglutination	Agglutination	B
Agglutination	Agglutination	AB
No agglutination	No agglutination	O

Rh AGGLUTINATION REACTION

Rh AGGLUTINATION FACTOR	Rh FACTOR
Agglutination	+
No agglutination	–

SUPPLIES NEEDED

- Anti-A serum (simulated)
- Anti-B serum (simulated)
- Anti-Rh serum (simulated)
- 1 blood-typing tray
- 1 suspect blood sample
- 3 stirring sticks
- Paper towels

DIRECTIONS

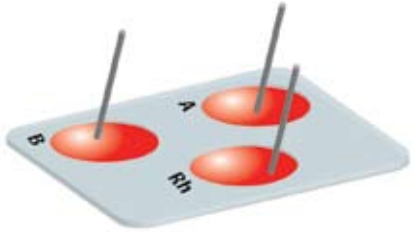
Group 1 will test blood from suspect 1.
Group 2 will test blood from suspect 2.
Group 3 will test blood from suspect 3.
Group 4 will test blood from suspect 4.

1. Place 2 drops of assigned suspect's blood evidence (simulated blood) into each well of the blood-typing tray.
2. Place 1 drop of anti-A simulated serum in Well A.
3. Next place 1 drop of anti-B simulated serum in Well B.
4. Finally place 1 drop of anti-Rh simulated serum in Well Rh.
5. Use a separate stirring stick to mix the simulated blood and serum in each well for 10 seconds.
6. Carefully examine each well to determine if the simulated blood in each well has clumped or agglutinated. Record your results in the correct place on the board.
7. Rinse the tray thoroughly and dry with the paper towel.

Put 2 drops of simulated blood into each well.

Put 1 drop of each anti-serum into the corresponding well.

Mix the blood and serum in each well for 10 seconds.



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DNA EVIDENCE

INSTRUCTIONS FOR TEACHERS

Cut on the dotted line to separate the 5 DNA sequences on page 46. **BE SURE TO RETAIN THE LABEL ON EACH STRIP**. Place each strip into a business envelope that is correctly labeled to match the source of the DNA.

Briefly describe the structure of DNA as it relates to the DNA samples. The 5' and 3' numbers are used to orient the strands. If you have the sequence of one strand, you automatically know the sequence of the other strand using base pair rules. For simplicity, we are using the sequence of one strand only.

Students should model the action of restriction enzymes by looking for the sequence "GAATTC." They are to cut the sequence after the "G."

Create an imaginary gel with the base pair numbers provided. Collect data from each group, and ask volunteers to tape their fragments in the correct places on the imaginary gel. You can eliminate suspects whose fragments do not match those of the crime scene.

DNA EVIDENCE

INSTRUCTIONS FOR STUDENTS

Work only with the DNA sample you have been assigned.

1. Recall the structure of DNA and the base pairing rules discussed earlier in class. Observe the DNA sample you have been provided.

2. The 5' and 3' numbers are used to orient the strands. If you have the sequence of one strand, you automatically know the sequence of the other strand using base pair rules.

3. Model the action of restriction enzyme HaeIII by looking for the sequence "GGCC." You are to mark and cut the sequence between the last G and the first C in the sequence each time you find "GGCC" in your assigned DNA sample.

4. Below each nitrogen base, write on the solid line the corresponding base pair. (Remember the base pairing rules: A pairs with T, and C pairs with G.)

5. Count the number of base pairs (bp) in each fragment of DNA that you have. Write the number of base pairs on the blank side of each fragment. Use a marker so that the number will be very visible.

CRIME SCENE

5’ GGCCGAATTCAGGCCAATTC 3’
3’ _____ 5’

- -

SUSPECT 1

5’ GGCCGAATTCAGGCCAATTC 3’
3’ _____ 5’

- -

SUSPECT 2

5’ GGAAGGCCATACGAGGGCCC 3’
3’ _____ 5’

- -

SUSPECT 3

5’ GGGCCTCATACGAATTGGCC 3’
3’ _____ 5’

- -

SUSPECT 4

5’ GGCCATTCATGGCCTGGCCA 3’
3’ _____ 5’

- -

KEY

CRIME SCENE

5’ GG | CCGAATTCAGG | CCAATTC 3’
3’ CC | GGCTTAAGTCC | GGTTAAG 5’
2 | 11 | 7

SUSPECT 1

5’ GG | CCGAATTCAGG | CCAATTC 3’
3’ CC | GGCTTAAGTCC | GGTTAAG 5’
2 | 11 | 7

SUSPECT 2

5’ GGAAGG | CCATACGAGGG | CCC 3’
3’ CCGGCC | GGTATGCTCCC | GGG 5’
6 | 11 | 3

SUSPECT 3

5’ GGG | CCTCATACGAATTGG | CC 3’
3’ CCC | GGAGTATGCTTAACC | GG 5’
3 | 15 | 2

SUSPECT 4

5’ GG | CCATTCATGG | CCTGG | CCA 3’
3’ CC | GGTAAGTACC | GGACC | GGT 5’
2 | 10 | 5 | 3

30	24	18	12	6
29	23	17	11	5
28	22	16	10	4
27	21	15	9	3
26	20	14	8	2
25	19	13	7	1

EXPLANATION/ELABORATION ACTIVITY

INSTRUCTIONS FOR TEACHERS

- Each group will complete their blood evidence and place the results on the board (ABO and Rh).
- Ask each group to place their DNA fragments on the board.
- Challenge each group to either support or refute the explanations given for each type of evidence.
- Lead a class discussion around the following topics:
    - DNA structure/models of DNA
    - Milestones in forensic DNA analysis
    - DNA fingerprints vs. actual fingerprints
    - Applications of DNA fingerprinting
    - Making a DNA fingerprint (PCR, restriction enzymes and gel electrophoresis)
    - Variations in DNA (mutations & VNTR)
    - Why is DNA testing sometimes controversial?
    - Validity of blood and DNA evidence

CLASS ANALYSIS OF EVIDENCE

Put a + in each block that matches with the evidence found at the crime scene.

	Blood evidence Blood type	Frequency of ABO Blood Type in the U.S.	Fiber Type	Fingerprints	Results of DNA Evidence
Evidence	A+	34%			
Suspect 1					
Suspect 2					
Suspect 3					
Suspect 4					

ELABORATION ACTIVITY

DNA structure/models of DNA

- X-ray diffraction; picture of DNA (Figure 1)

Milestones in forensic DNA analysis

- Students might assume that DNA testing has been around for a long time, but it only began in the 1980s.

DNA fingerprints vs. actual fingerprints

- Applications of DNA fingerprinting
  - ID victims of war or tragedy
  - Paternity cases
  - Thomas Jefferson case
  - Tomb of the Unknown Solider
  - Location of ancestors
  - Wildlife forensics
  - Darrell Hunt case
- First legal uses of DNA profiling (Dr. Alec Jeffries — British immigration case and Foot Path Murders)
- Other uses of DNA profiling
  - World Trade Center bombing
  - Barry Scheck — Innocence Project

Making a DNA fingerprint (PCR, restriction enzymes and gel electrophoresis)

- Label gel and describe its function
- Restriction enzymes/purpose
- How DNA evidence is collected by crime scene investigators
  - DNA is extracted from sources at the crime scene and from victims and suspects
- How are DNA samples processed to create a DNA profile?
  - PCR/RFLP isolation of DNA from RFLP analysis is very time consuming and labor intensive. PCR can be used to amplify very small amounts of DNA in 2-3 hours, to the levels required for RFLP analysis.
- What is the point of PCR?
  - To determine the genotype (DNA profile), crime-scene investigators make millions of copies of the target sequence using PCR.

Variations in DNA (mutations & VNTR)

- Humans have 99.9% similarity in their DNA
  - STR regions
  - Examples of STRs; determining genotypes for individuals using STRs
  - Crime scene investigators use techniques that are fast, cost effective, and have a high power of discrimination; the power of discrimination increases with the number of loci profiled
- STR Frequencies
  - STR frequencies vary in ethnic groups, increasing the power of discrimination
  - CODIS — A federally maintained database used by law enforcement officials
  - Analysis of Results: who can't be excluded?

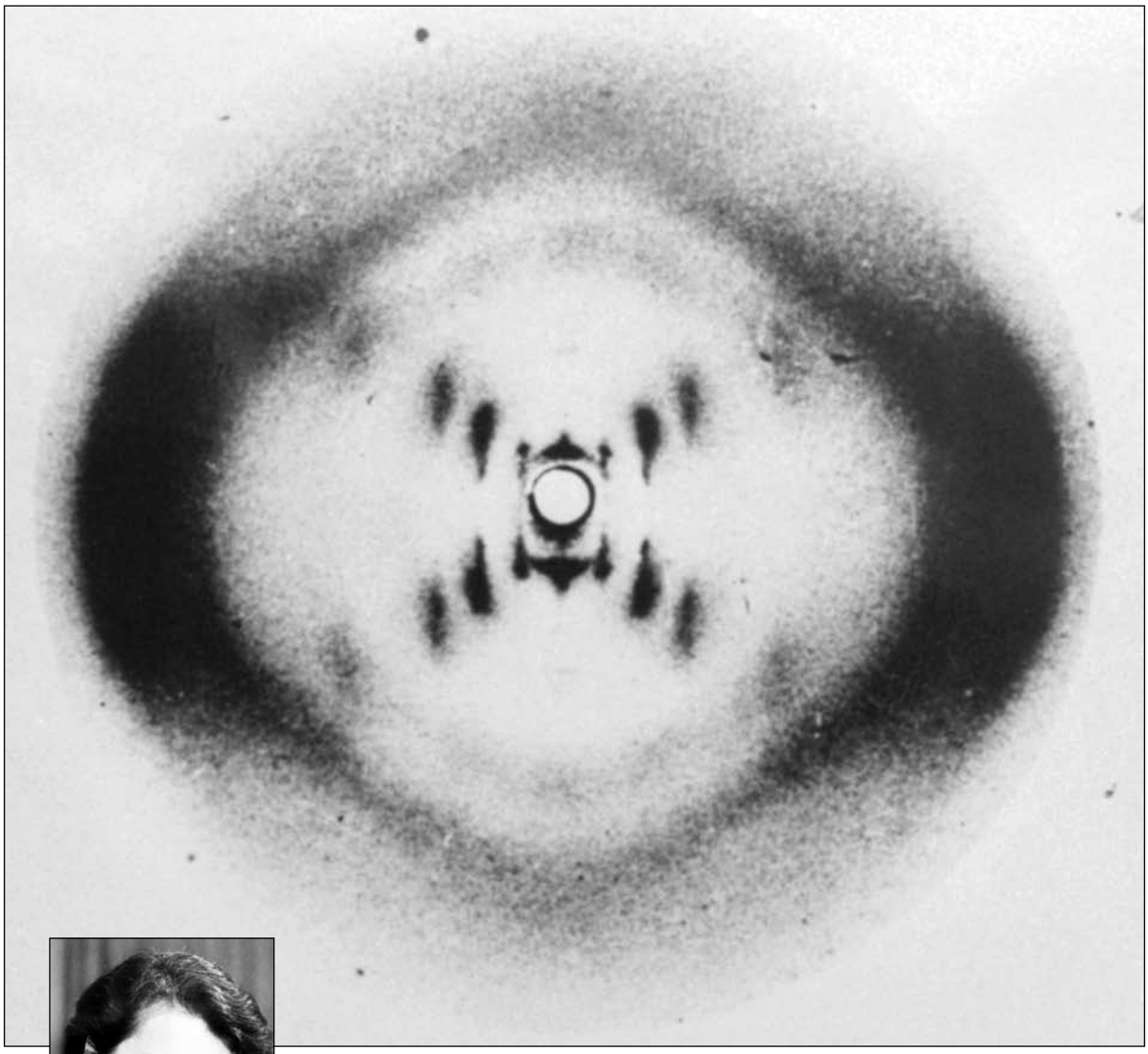
The power of discrimination

Why is DNA testing sometimes controversial?

- Chain of custody
  - In other words, DNA fingerprinting can show that a DNA sample is very likely to have come from a specific individual, but it does not show *how* the DNA arrived at the crime scene.
- Validity of blood and DNA evidence
  - Use of the product rule to calculate the probability of another person having the same DNA pattern as the suspect at multiple sites: the probability of multiple events occurring simultaneously is obtained by multiplying the probabilities for the occurrence of each event.

FIGURE 1: X-RAY DIFFRACTION PHOTOGRAPH OF DNA

This photo of DNA, taken by X-ray crystallographer Rosalind Franklin, enabled Francis Crick and James Watson to deduce DNA's double-helical structure.



Photos courtesy of the James D. Watson Collection, Cold Spring Harbor Laboratory Archives

Rosalind Franklin

FIGURE 2: DNA STRUCTURE

FIGURE 3: MILESTONES IN FORENSIC DNA ANALYSIS

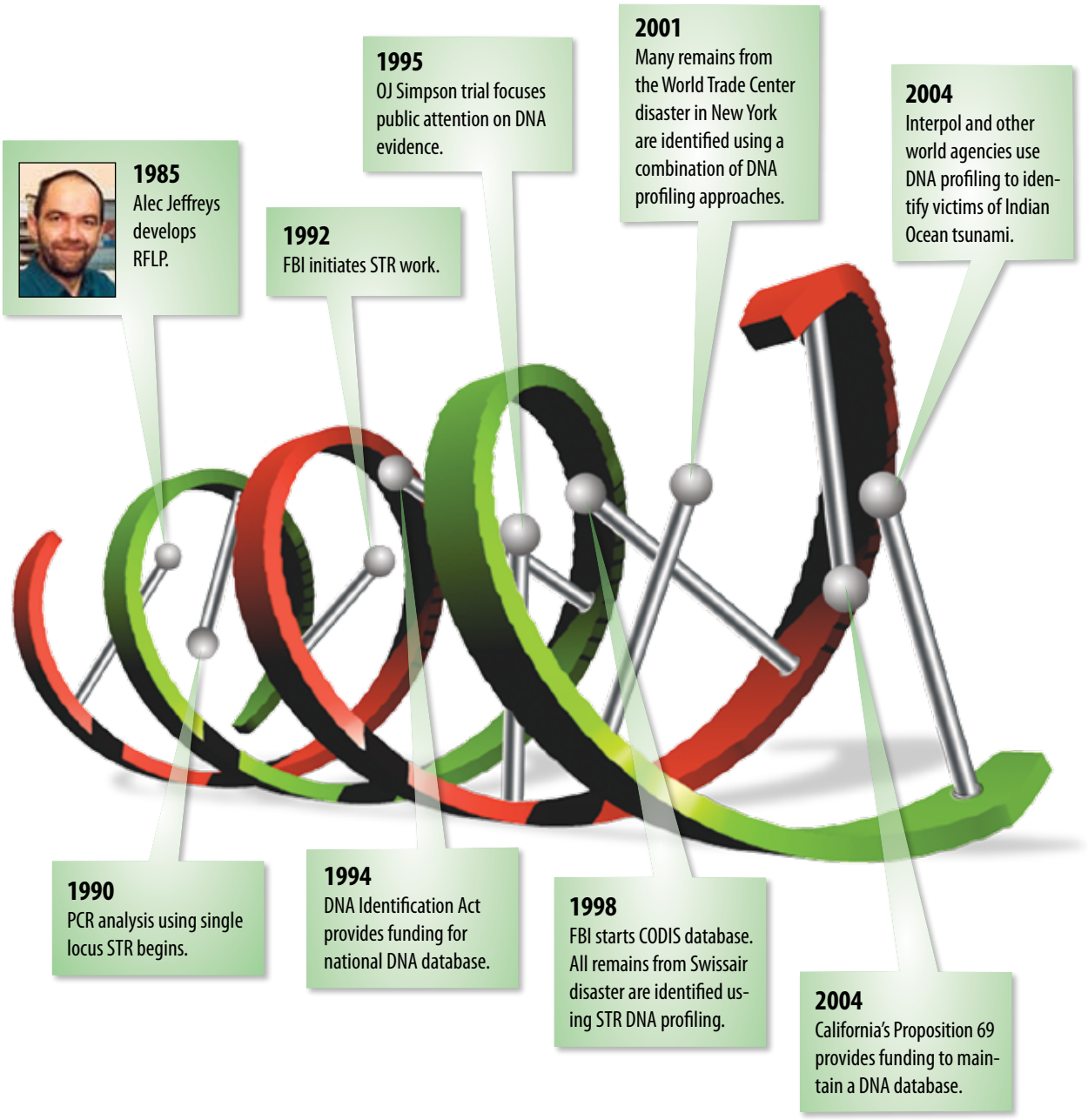
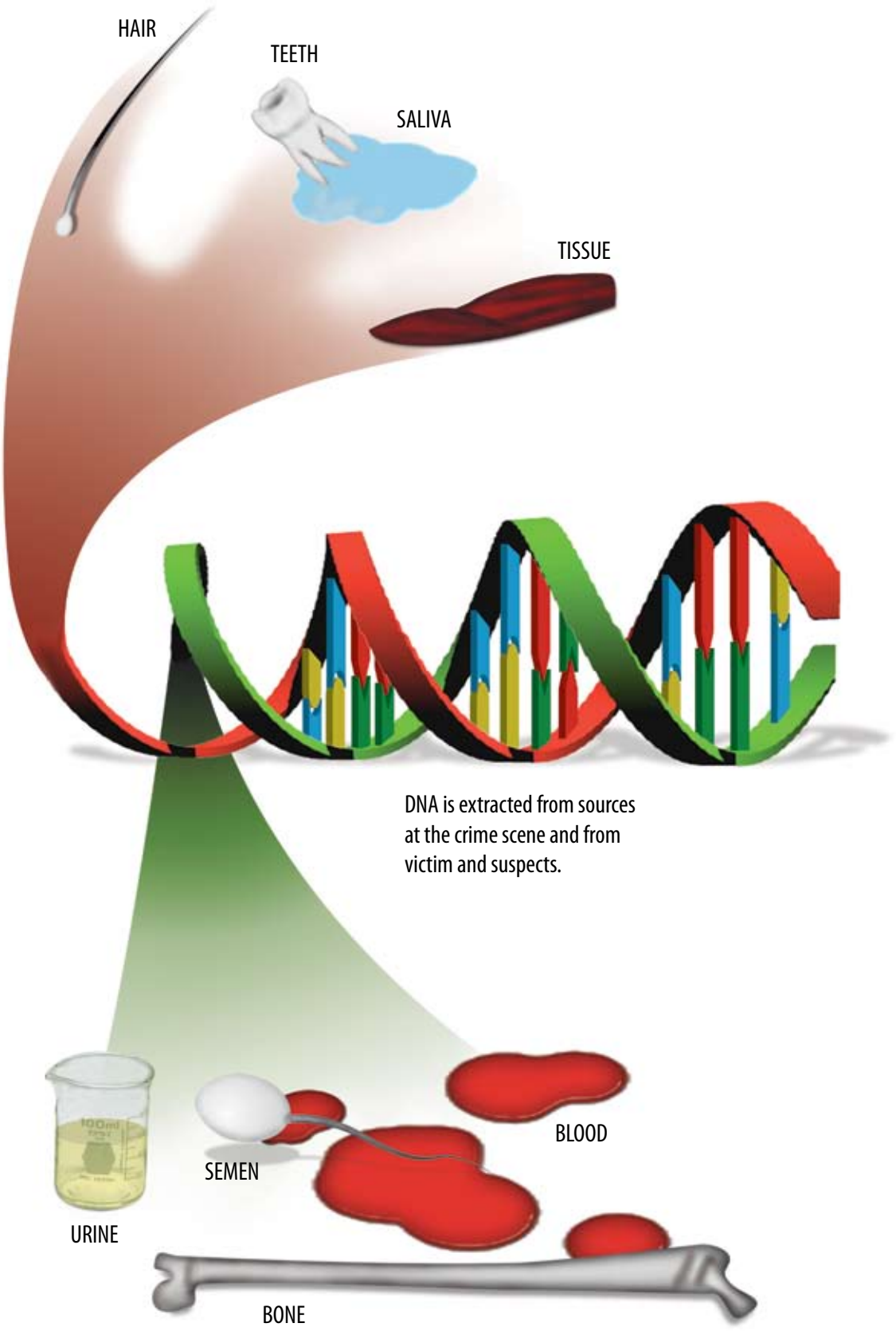


FIGURE 4: HOW DNA IS COLLECTED BY THE CRIME-SCENE INVESTIGATORS



RESTRICTION ENZYMES

Bacteria have restriction enzymes that break down the foreign DNA of invading viruses. These enzymes are helpful in "cutting" DNA into fragments. Examine the results as the restriction enzyme EcoRI makes staggered cuts at specific sites along this strand of DNA:

```
5′   A T G A A T T C T  G A A T T C C C T   3′
3′   T A C T T A A G A  C T T A A G G G A   5′

5′   A T G          A A T T C T G          A A T T C C C T 3′
3′   T A C T T A A        G A C T T A A          G G G A 5′
```

1. The number of fragments of DNA that result from those cuts is _____.

2. What do we call the chemical that cuts the DNA?

3. The restriction enzyme used above cuts DNA everywhere the base pattern _____ is found.

4. The restriction enzyme called HaeIII cuts DNA at the following sequence:

```
CCGG
GGCC
```

It cuts between the C and the G and produces blunt cuts. Indicate the DNA fragments that would result if HaeIII was used to cut the DNA fragment below:

```
T A C C G G G A A T T C A T C C G G T G A A T T
A T G G C C C T T A A G T A G G C C A C T T A A
```

5. What differences are there in the fragments?

6. We can express the DNA fragments as the number of base pairs in each fragment. How many base pairs (bp) are found in the smaller fragment?

7. The words EYE, POP, DID, RACECAR, and RADAR are palindromes. What are palindromes, and what do they have to do with restriction enzymes?

8. Do you think restriction enzymes could be used to cut DNA from organisms other than humans? Explain.

RESTRICTION ENZYMES

KEY

Bacteria have restriction enzymes that break down the foreign DNA of invading viruses. These enzymes are helpful in "cutting" DNA into fragments. Examine the results as the restriction enzyme EcoRI makes staggered cuts at specific sites along this strand of DNA:

```
5′    A T G A A T T C T  G A A T T C C C T        3′
3′    T A C T T A A G A   C T T A A G G G A        5′

5′    A T G              A A T T C T G            A A T T C C C T 3′
3′    T A C T T A A                 G A C T T A A            G G G A 5′
```

1. The number of fragments of DNA that result from those cuts is 3.

2. What do we call the chemical that cuts the DNA?

   Restriction enzyme

3. The restriction enzyme used above cuts DNA everywhere the base pattern GAATTC is found.

4. The restriction enzyme called HaeIII cuts DNA at the following sequence:

   ```
   CCGG
   GGCC
   ```

   It cuts between the C and the G and produces blunt cuts. Indicate the DNA fragments that would result if HaeIII was used to cut the DNA fragment below:

   ```
   T A C C | G G G A A T T C A T C C | G G T G A A T T
   A T G G | C C C T T A A G T A G G | C C A C T T A A
   ```

5. What differences are there in the fragments?

   The fragments are different lengths.

6. We can express the DNA fragments as the number of base pairs in each fragment. How many base pairs (bp) are found in the smaller fragment?

   Four.

7. The words EYE, POP, DID, RACECAR, and RADAR are palindromes. What are palindromes, and what do they have to do with restriction enzymes?

   Palindromes are words that have the same spelling forward as well as backwards. Restriction enzymes cut DNA at specific restriction sites that have the same sequence of nitrogen bases, backwards and forwards.

8. Do you think restriction enzymes could be used to cut DNA from organisms other than humans? Explain.

   Yes, restriction enzymes could be used to cut DNA from other organisms. The nitrogen bases (A,T,C,G) are the same for all living organisms.

GEL ELECTROPHORESIS

Electrophoresis works on the premise that different-sized DNA fragments move at different rates through a gel matrix. Long DNA fragments with many base pairs have more bulk and move more slowly than small pieces of DNA.

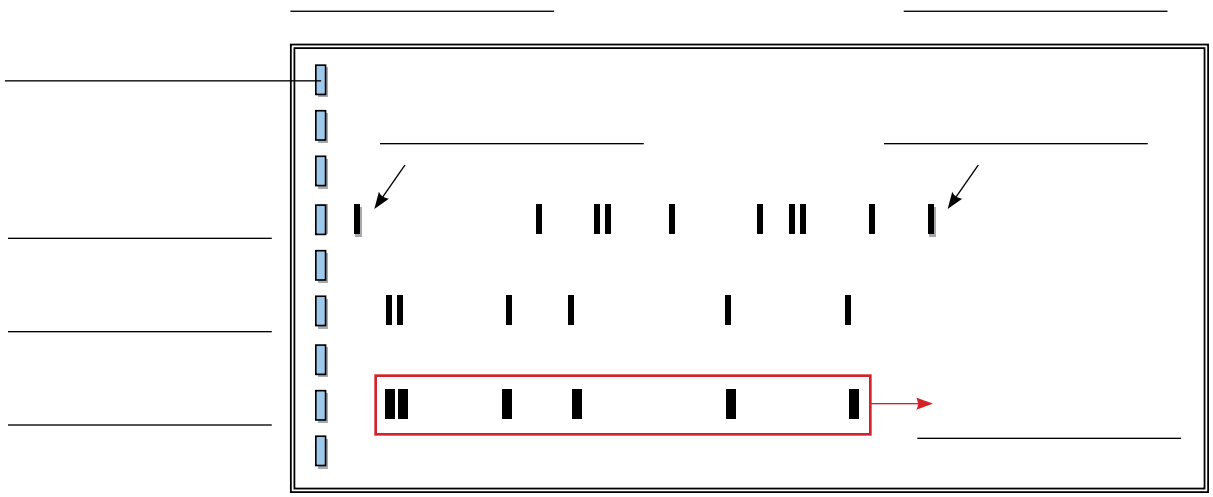
As the name "electrophoresis" suggests, an electric current drives the migration of DNA from the negative end to the positive end of the gel. In this case, the negatively charged DNA fragment is placed in the wells at the negative end (left side) of the gel and moves toward the positive end (right side) of the gel. The gel serves as a sieve to separate out the DNA fragments based on their sizes.

Below, the crime scene DNA is placed in the well second from the bottom of the gel and makes a DNA fingerprint. Suspect 1's DNA is placed in the fourth well from the bottom, and Suspect 2's DNA is placed in the sixth well up from the bottom.

After a DNA sample finishes moving through the gel, a distinct banding pattern shows where the different DNA fragments have ended up. In this way, the fragments are sorted according to their sizes. The banding pattern that results from this is a unique DNA fingerprint that, like the fingerprints on one's hand, differs from one person to another. (The exceptions are identical siblings, such as twins or triplets, who share the same genetic fingerprints.)

Identify the following parts of the gel shown below:

Positive end	Negative end
Largest DNA fragment	Smallest DNA fragment
DNA fingerprint	Wells
Crime-scene DNA	Suspect 1's DNA
Suspect 2's DNA	

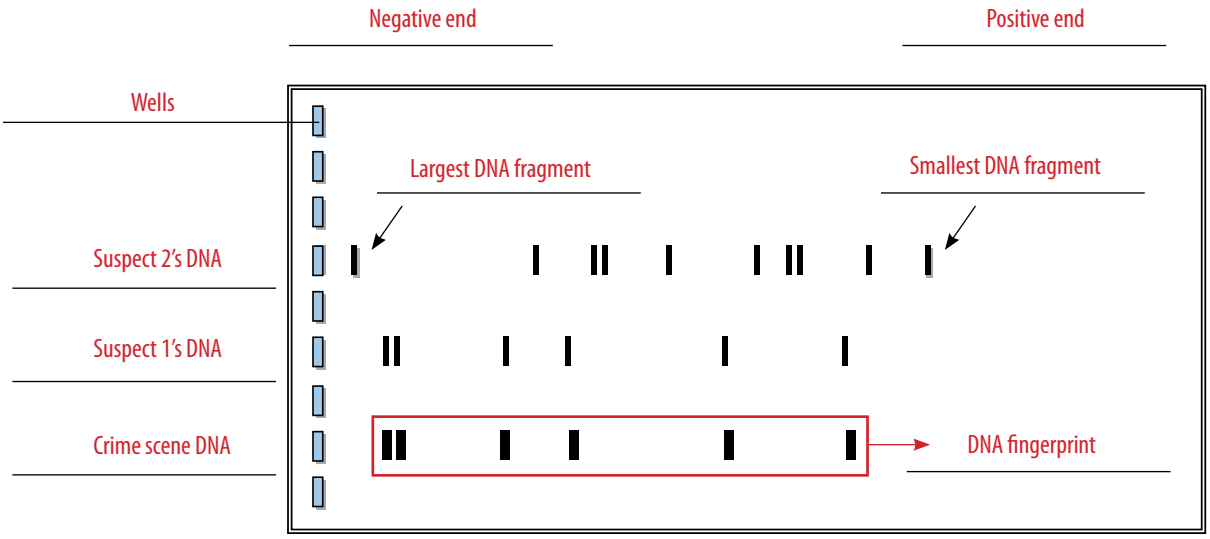


WHO CAN'T BE EXCLUDED?

- Suspects are included in an investigation if their DNA profiles match with genotypes found at the crime scene.
- Suspects can be excluded if their DNA profiles do not match genotypes found at the crime scene.

GEL ELECTROPHORESIS

KEY

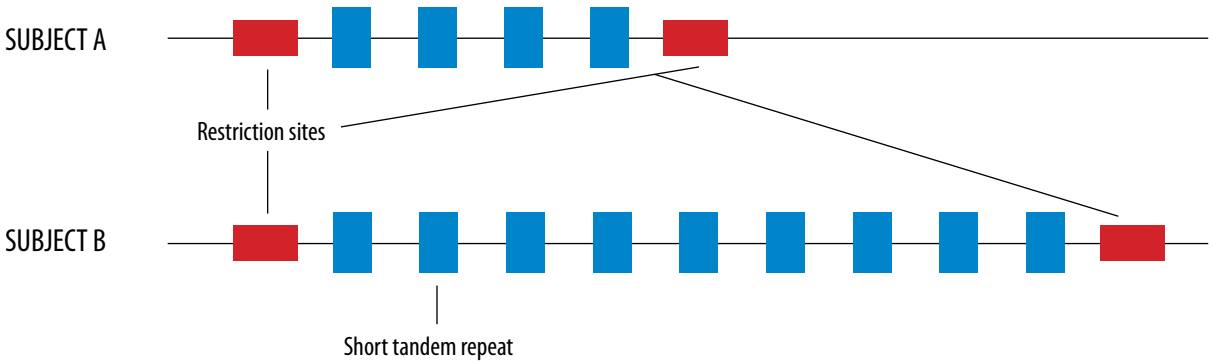


SHORT TANDEM REPEATS (STRs)

Since humans are 99.9% identical, where do crime scene investigators look for differences in DNA profiles?

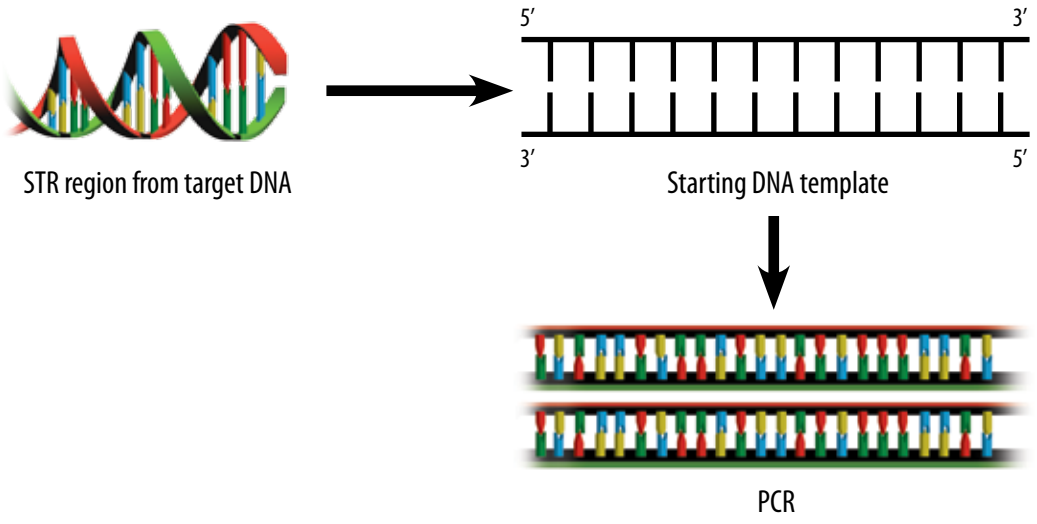
There are areas of DNA that are unique from individual to individual and are "anonymous," which means they control no known trait or function. The areas examined are short tandem repeats (STRs). A tandem repeat is a short DNA sequence that is repeated in a head-to-tail fashion at a specific chromosomal locus. For many tandem repeats, the number of repeated units vary between individuals. These are called variable number of tandem repeats (VNTRs). The size of restriction fragments will vary according to the number of tandem repeats.

FIGURE 5: VARIABLE NUMBER OF TANDEM REPEATS (VNTRs)



POLYMERASE CHAIN REACTION (PCR)

To determine the genotype (DNA profile), crime-scene investigators make billions of copies of the target sequence using PCR (polymerase chain reaction).



EXAMPLES OF STR

The TH01 locus contains repeats of TCAT.

CCC TCAT TCAT TCAT TCAT TCAT TCAT AAA

This example has six TCAT repeats.

There are more than 20 known TH01 alleles.

Each individual inherits one allele from each parent.

DETERMINING GENOTYPES FOR INDIVIDUALS USING STRs

Ms. Smith's TH01 locus for her two chromosomes is given below. What is her genotype?

Mom's chromosome:

CCC TCAT TCAT TCAT TCAT TCAT TCAT AAA

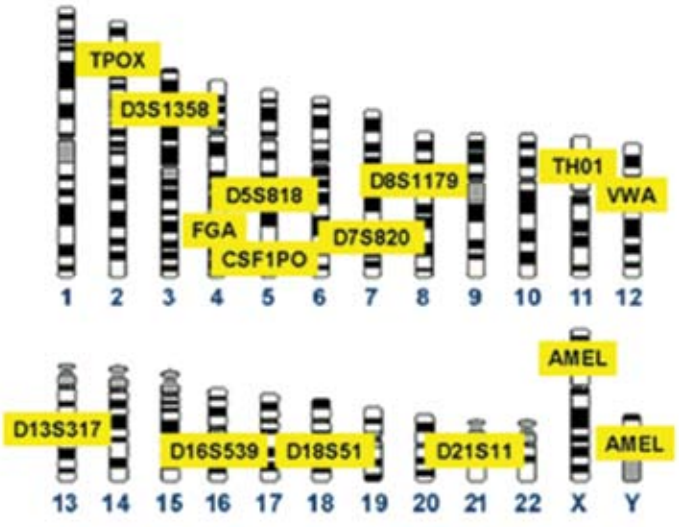
Dad's chromosome:

CCC TCAT TCAT TCAT TCAT TCAT TCAT TCAT TCAT TCAT TCAT TCAT TCAT TCAT TCAT AAA

CODIS

Combined DNA Index System — a federally maintained database used by law-enforcement officials.

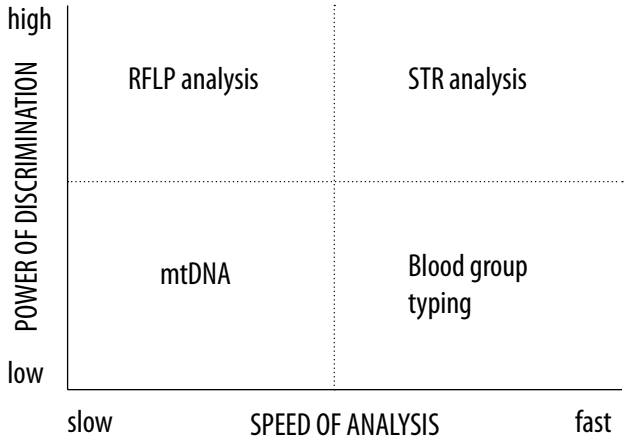
13 CODIS core STR loci with chromosomal positions



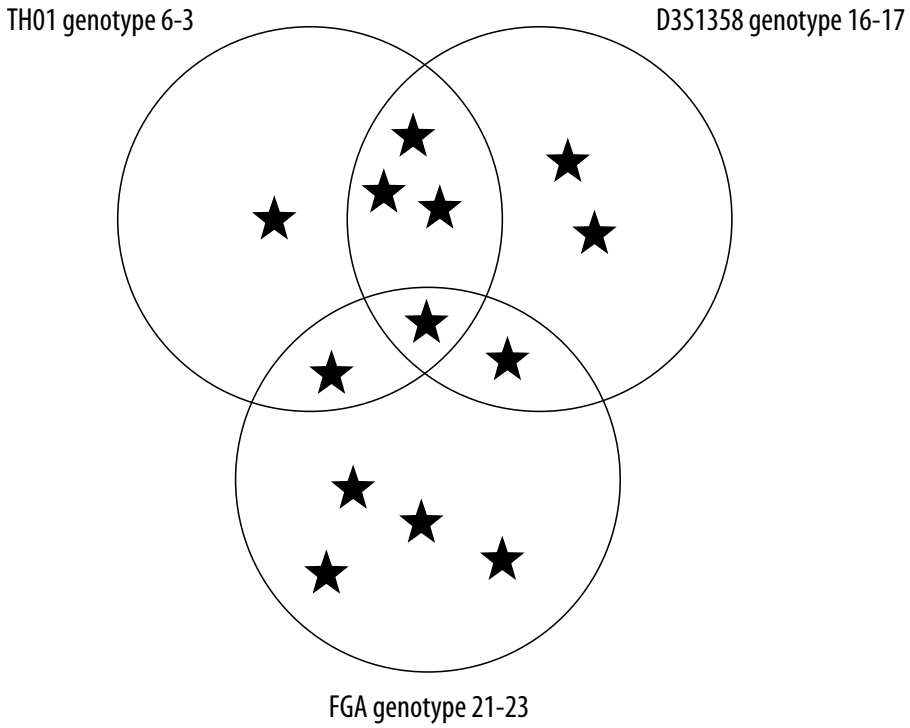
13 loci guarantee high power of discrimination

POWER OF DISCRIMINATION

Crime-scene investigators use techniques that are fast and cost-effective and have a high power of discrimination.



The power of discrimination increases with the number of loci profiled.



THE POWER OF DISCRIMINATION

DNA fingerprinting is powerful because of the combined analysis of a number of VNTR loci located on different chromosomes. The term VNTR (variable number of tandem repeats) refers to the variable sequence rather than the method used to detect it. RFLP is used to detect the variability in the number of repeats. Several PCR-based methods can be used to detect VNTRs. PCR has supplanted RFLP by southern blotting and hybridization in analysis of VNTRs.

- We cannot pinpoint a suspect with the results of only one VNTR locus any more than we can identify a person by just one digit from his or her social security number.

- A DNA profile is compiled from the results of four or five probes that are applied sequentially to a membrane, each probe targeting a different VNTR locus.

- Using four probes actually provides eight pieces of information about an individual, since each person has two separate copies of each VNTR region. (*To get the probability that a given 8-band profile will occur, multiply the eight different allele frequencies together.*)

- Each VNTR locus has about 30 different lengths variants (alleles).

Each allele occurs at a certain frequency within a given population. The frequency of occurrence of different VNTR alleles in many different populations of racially and ethnically diverse peoples has been determined. *Frequencies vary in ethnic groups, increasing the power of discrimination.*

THE FREQUENCY OF A FIVE-LOCI DNA PROFILE

Imagine that a DNA forensic scientist determined that DNA from semen from a vaginal swab of a rape victim matched the DNA profile of a suspect at five different VNTR loci. Also assume that the frequency of the DNA profiles for the 5 individual loci were 0.01, 0.02, 0.06, 0.10, and 0.03. How common or rare would this 5 locus DNA profile be in the reference population?

In most cases, a "product rule" calculation can be done by multiplying each individual probability together. Thus, the frequency of the profile is 0.01x 0.02 x 0.06 x 0.10 x 0.03= 3.6E-08.

Another way to express this probability is the reciprocal of this number: 1/3.6E-08=27.8 million. *The DNA analyst could report that the DNA profile that is shared by the suspect and the evidence might occur by chance in 1 person out of 27.8 million. The jury could use this information to evaluate whether the match of DNAs might have occurred by chance.*

The number of repeats at a single VNTR locus can't distinguish an individual from the rest of the population. The combined results from a number of loci produce a pattern unique to that person.

The FBI incorporates 13 sites on average into its profiles. With 26 different bands studied, it would be almost impossible to find two unrelated individuals with the same DNA profile. The odds of a match in this case are well more than one in a hundred billion. (The U.S. population is 300 million. The world population is more than 6 billion.)

CODIS (Combined DNA Index System) 13 loci guarantee high power of discrimination

How are suspects included or excluded from an investigation?

- Suspects are **included** in an investigation if their DNA profiles match with genotypes found at the crime scene.
- Suspects can be **excluded** if their DNA profiles do not match genotypes found at the crime scene.

CALCULATING A DNA PROFILE FREQUENCY

Locus	Locus Frequency	Combined Frequency	Combined Frequency Expressed as the Reciprocal 1/Combined Frequency
1	0.003 or 1 in 333	————	————
2	0.012 or 1 in 83		
3	0.010 or 1 in 100		
4	0.040 or 1 in 25		
5	0.1616 or 1 in 19.15		

1. Five different loci were used for this study. What is the total number of number of different bands studied? How did you determine your answer?

2. Would these five loci be enough to exclude this individual if the crime occurred in the U.S.? Explain your answer.

REFERENCES

Crime Scene Investigator PCR Basics(TM) Kit. Retrieved August 31, 2006, from Bio-Rad Laboratories Web site: http://www.bio-rad.com/LifeScience/docs/Official_Crime_Scene_PowerPoint_Spring_2005_rev_B.ppt#257,2,Crime Scene Investigator PCR Basics™ Kit

Meeker-O'Connell, Ann How DNA Evidence Works. Retrieved July 27, 2006, from howstuffworks Web site: http://science.howstuffworks.com/dna-evidence4.htm

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KEY

CALCULATING A DNA PROFILE FREQUENCY

Locus	Locus Frequency	Combined Frequency	Combined Frequency Expressed as the Reciprocal 1/Combined Frequency
1	0.003 or 1 in 333	————	————
2	0.012 or 1 in 83	0.003 x 0.012 $= 3.6 \times 10^{-5}$	1 in 27,777
3	0.010 or 1 in 100	0.003 x 0.012 x 0.01 $= 3.7 \times 10^{-7}$	1 in 2,777,777
4	0.040 or 1 in 25	0.003 x 0.012 x 0.010 x 0.040 = 1.44×10^{-8}	1 in 6.9 billion
5	0.1616 or 1 in 19.15	0.003 x 0.012 x 0.010 x 0.040 x 0.1616 = 2.33×10^{-9}	1 in 430 billion

1. Five different loci were used for this study. What is the total number of different bands studied? How did you determine your answer?

Using five probes actually gives you ten pieces of information about an individual since each of us has two separate copies of each VNTR region. To get the probability that a given 10 band profile will occur, you multiply the five loci frequencies together.

2. Would these five loci be enough to exclude this individual if the crime occurred in the U.S.? Explain your answer.

Yes, provided the individual does not have an identical twin. The population of the US is far less than 1 in 430 billion about 300 million. The DNA analyst could report that the DNA profile that is shared by the suspect and the evidence might occur by chance in 1 person out of 4.3 million.

EVALUATION: INCRIMINATING EVIDENCE

Write a paragraph that gives a possible scenario as to what might have happened based on the evidence and describe how the physical evidence supports your hypothesis. Use as many of the terms provided as possible.

agglutination
agarous gel
blunt cut
comb
CODIS
DNA restriction analysis
DNA fingerprinting
DNA fragments (RFLP)
digital micropipet
electric field
exclusion
evidence
fingerprint
gel electrophoresis
gel lanes
gene
human genome
inclusion
luminol
nucleotides
physical evidence
palindrome
PCR
restriction digest
restriction enzymes
restriction site
RFLP
ridge characteristics
staggered cut
STR
VNTR

GET A CLUE IMPLEMENTATION PLAN — WET-LAB

Activity	Estimated Time	Materials/Equipment	Purpose/Objectives/ Essential Question
Scenario similar to that of the pre-lab	5 minutes	3 micro centrifuge tubes of DNA from the crime scene or Bio-Rad's Forensic DNA Fingerprinting Kit 16-0007EDU	**Purpose:** To apply the concepts of restriction enzymes, electrophoresis, and DNA restriction analysis **Objectives:** • To build confidence in the students' ability to do science • To model the process of restriction analysis • To identify a need for DNA restriction analysis • To apply DNA restriction analysis to the identification of DNA fragments • To communicate in writing the meaning of the results for the case studied • To develop skills in using pipets, electronic balances, electrophoresis boxes **Essential Question:** Who is guilty of the crime based on the results of gel electrophoresis of the DNA found at the crime scene?
Role play	5 minutes	Large cardboard scissors DNA sequences on colored paper to represent nucleotide sequences	
Practice Using Micropipets	10 minutes	Student Stations: Micropipets/tips Micro centrifuge tubes with green and blue food coloring 1 15ml tube Student copies — Practice with P20 & P200	
Practice loading gels	10 minutes	Student Stations: Micropipets with Tips Practice loading stations with loading dye	
Preparation of the gel	15 minutes	Balance, vortex, hot water baths Each student gets: .45 grams agarose 32 ml buffer 1 20 X 200 test tube Gel tray and Comb Graduated Cylinder	
Electrophoresis of DNA taken from crime scene	25 minutes	Electrophoresis chamber and power supply 500 ml of DNA buffer Samples of DNA from the crime scene in laptop coolers Micropipets with tips	
Analysis of DNA from crime scene	5 minutes	Carolina Blu Stain Staining Tray Light Box Final Report Form	

Alignment with NC Competency Goals

Biology

Goal 1 Objectives 1.01, 1.02, 1.03, 1.04, 1.05 Goal 2 Objectives 2.01, 2.02, 2.04	Goal 3 Objectives 3.01, 3.04

WET-LAB ENGAGEMENT ACTIVITY

MATERIALS NEEDED

- Get a Clue Worksheet for each student
- Four Cards with the DNA Sequences (copy on card stock paper)

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

- Large cardboard scissors labeled "Restriction Enzyme" on the blades
- Brad/fastener for the scissors and tape to secure the fastener

ENGAGEMENT ACTIVITY

1. The teacher should ask for four volunteers to be participants in the DNA role play.

2. The teacher will provide each volunteer with a segment of the DNA sequence above. Each of the four students will be asked to stand close to each other, locking arms, and hold their fragment up so that all four cards are connected to form a continuous strand of DNA.

Now is an excellent time to review DNA structure using the "Get a Clue Worksheet."

3. Next, the teacher should give the cardboard scissors to another class member and ask that student to cut between the bases T and C. There should be :

- 2 four-nucleotide pieces
- 1 three-nucleotide piece
- 1 one-nucleotide piece

Ask the students to consider the following questions:

> Imagine the DNA cut up in a test tube. Could you distinguish the different size pieces?
>
> How could we make the pieces distinguishable?
>
> Earlier in the pre-lab activities you physically manipulated paper models of DNA fragments to arrange them by size. You are now faced with the problem of arranging very small fragments of DNA molecules without being able to manipulate them with your hands. How can this be done?
> By the process of gel electrophoresis.

4. Remind students they have most likely dealt with a similar problem before, playing in a sand box, sifting large pieces of dirt from small pieces of dirt.

> What might you use to separate the large pieces of dirt from the smaller ones in a sandbox?
> A sifter or sieve.

5. The same problem exists with fragments of DNA that need to be separated. A sieve with very small holes can be used to separate the fragments.

> Imagine the room is a gel and the DNA molecules are at one end. What do the spaces between the chairs represent?
> The spaces in the gel.
>
> In the sand box analogy, what force pulled the sand trough the sieve?
> Gravity.
>
> Gravity will not be effective on the small, negatively charged fragments of DNA. What would happen if we put them in an electric field with a positive end and a negative end?
> The negatively charged DNA fragments would migrate to the positive pole.

6. Tell students to imagine a negative electrode at one end of the room and a positive electrode at the opposite end of the room. What would happen when the electricity was turned on?

> Which DNA fragment would move faster through the gel?
> The smaller one.

7. Ask students to walk through the process.

> Have the fragments been separated by size?
> Yes

5'

A

G

T

3'

T

A

C

G C

T A

C G

A T

G C

C G

T A

A T

AGAROSE GEL ELECTROPHORESIS AND VISUALIZATION OF DNA FRAGMENTS

From Bio-Rad's Forensic DNA Fingerprinting Kit Instruction Manual

ADVANCE PREPARATION

Objectives

• Prepare HindIII lambda digest (DNA marker) and aliquot (optional)
• Aliquot sample DNA loading dye (optional)
• Prepare the electrophoresis chamber
• Dilute Fast Blast™ stain to 1x (for overnight staining) or 100x (for quick staining)
• Set up student and teacher workstations

Time required

45 minutes

Materials needed

• HindIII lambda digest (DNA marker)
• Sample loading dye
• Electrophoresis chambers, casting trays, and combs
• Electrophoresis buffer (1x TAE)
• Fast Blast DNA stain, 500x

Procedures

1. Prepare *Hin*dIII lambda digest (DNA marker) and aliquot (optional). Add 20 µl of sample loading dye to the stock tube containing the *Hin*dIII lambda DNA marker. If possible, heat the marker to 65°C for five minutes, then chill on ice — this results in better separation of the marker bands. Label clear micro test tubes "M". Aliquot 15 µl of the DNA markers containing loading dye to eight clear micro test tubes labeled "M".

2. Aliquot sample loading dye. Label eight clean micro test tubes "LD" for loading dye and aliquot 50 µl of sample loading dye into each tube. Distribute one tube to each team.

3. Prepare the electrophoresis chamber. When the agarose gel has solidified, sample loading and electrophoresis can begin.

   a. When placing the gel tray into the electrophoresis chamber, make sure that the sample wells are at the black cathode end. DNA samples will migrate toward the red anode end during electrophoresis.

   b. Prepare the required volume of 1x TAE buffer, if you have not prepared it already.

   c. Submerge the gel under about 2 mm of 1x TAE buffer.

   d. Prepare samples for gel loading. See laboratory protocol in the student section.

Note: Power requirements vary depending on gel thickness, length, and concentration, and on type of electrophoresis buffer used. For this exercise we recommend using a constant voltage of 100 V for 30 minutes.

4. Prepare Fast Blast DNA stain

   a. To prepare 100x stain (for quick staining), dilute 100 ml of 500x Fast Blast with 400 ml of distilled or deionized water in an appropriately sized flask or bottle and mix. Cover the flask and store at room temperature until ready to use.

   b. To prepare 1x stain (for overnight staining), dilute 1 ml of 500x Fast Blast with 499 ml of distilled or deionized water in an appropriately sized flask or bottle and mix. Cover the flask and store at room temperature until ready to use.

MAKING DNA VISIBLE

Fast Blast DNA stain is a convenient, safe, and nontoxic alternative to ethidium bromide for the detection of DNA in agarose gels following electrophoresis. Fast Blast contains a cationic compound that is in the thiazin family of dyes. The positively charged dye molecules are attracted to and bind to the negatively charged phosphate groups on DNA molecules. The proprietary dye formula stains DNA deep blue in agarose gels and provides vivid, consistent results.

Fast Blast DNA stain is provided as a 500x concentrate that must be diluted prior to use. The stain can be used as a quick stain when diluted to 100x to allow the visualization of DNA within 12–15 minutes or used as an overnight stain when diluted to 1x. When the agarose gel is immersed in Fast Blast DNA stain, the dye molecules attach to the DNA molecules trapped in the agarose gel. When the DNA bands are visible, your students can compare the DNA restriction patterns of the different samples of DNA.

Detailed instructions on using Fast Blast are included in the student manual.

WARNING

Although Fast Blast DNA stain is nontoxic and noncarcinogenic, latex or vinyl gloves should be worn while

handling the stain or stained gels to keep hands from becoming stained blue. Lab coats or other protective clothing should be worn to avoid staining clothes. Dispose of the staining solutions according to protocols at your facility. Use either 10% bleach solution or a 70% alcohol solution to remove Fast Blast from most surfaces. Verify that these solutions do not harm the surface prior to use.

NOTE

- We recommend using 120 ml of diluted Fast Blast to stain two 7 x 7 cm or 7 x 10 cm agarose gels in individual staining trays provided in the kit (you may want to notch gel corners for identification). If alternative staining trays are used, add a sufficient volume of staining solution to completely submerge the gels.

- Following electrophoresis, agarose gels must be removed from their gel trays before being placed in the staining solution. This is easily accomplished by holding the base of the gel tray in one hand and gently pushing out the gel with the thumb of the other hand.

- Because the gel is fragile, special attention must be given when handling it. We highly recommend using a large spatula or other supportive surface to transfer the gel from one container to another during the destaining steps involved with the quick staining protocol.

- Destaining (when performing the quick staining protocol) requires the use of at least one large-volume container, capable of holding at least 500 ml, at each student workstation. Each student team may utilize separate washing containers for each wash step, or simply use a single container that is emptied after each wash and refilled for the next wash.

- 100x Fast Blast can be reused at least seven times.

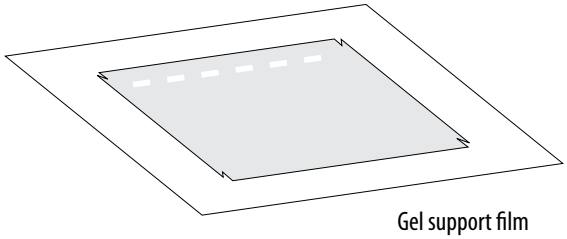
- No washing or detaining is required when using the overnight staining protocol.

To obtain a permanent record of the gel before it is dried, either trace the gel outline (including wells and DNA bands) on a piece of paper or acetate, take a photograph using standard cameras and film (Bio-Rad's standard Polaroid gel documentation system, catolog #170-3742EDU), or photocopy the stained gel.

DRYING THE AGAROSE GEL AS A PERMANENT RECORD OF THE EXPERIMENT

Note: Drying agarose gels requires the use of Bio-Rad's specially formulated high-strength analytical grade agarose. Other gel media may not be appropriate for this purpose.

We recommend using Bio-Rad's exclusive gel support film (catalog #170-2984EDU) to dry agarose gels. Remove the stained agarose gel from its staining tray and trim away any unloaded lanes with a knife or razor blade. Place the gel directly upon the hydrophilic side of a piece of gel support film. (Water will form beads on the hydrophobic side but will spread flat on the hydrophilic side of the film.) Center the gel on the film and remove bubbles that may form between the gel and film. Place the film on a paper towel and let the gel dry, making sure to avoid direct exposure to light. As the gel dries it will bond to the film but will not shrink. If left undisturbed on the support film, the gel will dry completely at room temperature after 2-3 days. The result will be a flat, transparent, and durable record of the experiment.



Gel support film

Note: Avoid extended exposure of dried gels to direct light to prevent band fading. However, DNA bands will reappear if the dried gels are stored in the dark for 2–3 weeks after fading.

GRAPHING THE DATA

Many of your students may not be familiar with logarithms and semilog graph paper. It is suggested that you prepare a short lesson to demonstrate the proper way to label the coordinates and plot the points. You might also choose to discuss the advantage of using semilog vs. standard graph paper in this instance. A math extension here can also provide an opportunity to explore linear and exponential (arithmetic and geometric) sequences of numbers.

QUICK GUIDE FOR DNA FINGERPRINTING KIT

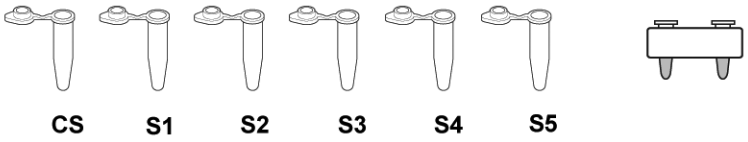
From Bio-Rad's Forensic DNA Fingerprinting Kit Instruction Manual

Restriction Digestion

1. Place the tube containing the restriction enzyme mix, labeled ENZ, on ice.

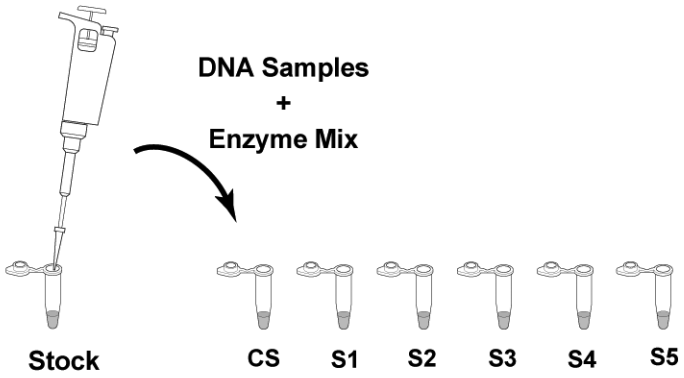
2. Label one of each colored micro test tubes as follows:

green tube CS	(crime scene)
blue tube S1	(suspect 1)
orange tube	S2 = suspect 2
violet tube	S3 = suspect 3
red tube	S4 = suspect 4
yellow tube	S5 = suspect 5



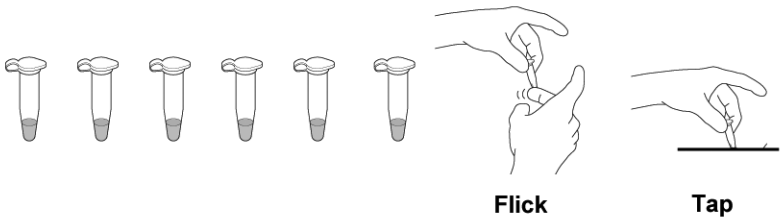
Label the tubes with your name, date, and lab period. Place the tubes in the foam micro test tube holder.

3. Using a fresh tip for each sample, pipet 10 µl of each DNA sample from the stock tubes and transfer to the corresponding colored micro test tubes. Make sure the sample is transferred to the bottom of the tubes.

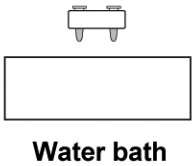


4. Pipet 10 µl of enzyme mix (ENZ) into the very bottom of each tube. Use a fresh tip to transfer the ENZ sample to each tube.

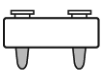
5. Tightly cap the tubes and mix the components by gently flicking the tubes with your finger. If a microcentrifuge is available, pulsespin in the centrifuge to collect all the liquid in the bottom of the tube. Otherwise, gently tap the tube on the table top.



6. Place the tubes in the foam micro tube holder and incubate for 45 minutes at 37°C or overnight at room temperature in a large volume of water heated to 37°C.

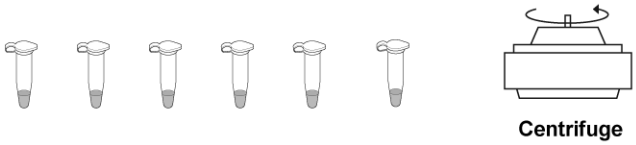


7. After the incubation period, remove the tubes from the water bath and place in the refrigerator until the next laboratory period. If there is sufficient time to continue, proceed directly to step 2 of agarose gel electrophoresis lesson.



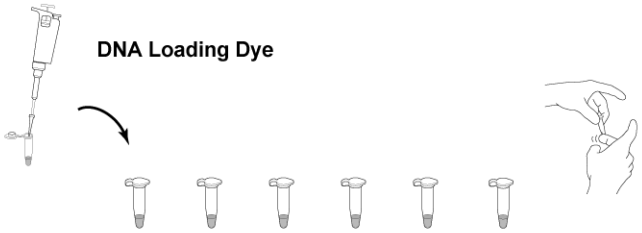
Agarose Gel Electrophoresis

1. Remove the digested DNA samples from the refrigerator (if applicable).



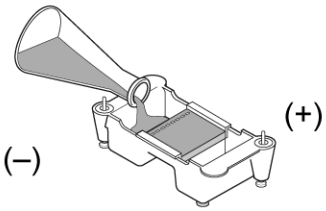
2. If a centrifuge is available, pulse spin the tubes in the centrifuge to bring all of the liquid into the bottom of the tube or gently tap on the table top.

3. Using a separate tip for each sample, add 5 µl of loading dye "LD" into each tube. Cap the tubes and mix by gently flicking the tube with your finger. Collect the sample at the bottom of the tube by tapping it gently on the table or by pulse-spinning in a centrifuge.



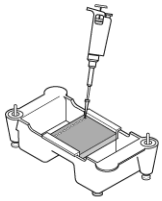
4. Remove the agarose gel from the refrigerator (if applicable) and remove the plastic wrap.

5. Place an agarose gel in the electrophoresis apparatus. Fill the electrophoresis chamber with 1x TAE buffer to cover the gel, using approximately 275 ml of buffer.



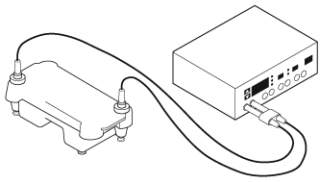
6. Check that the wells of the agarose gels are near the black (–) electrode and the bottom edge of the gel is near the red (+) electrode.

7. Using a separate tip for each sample, load the indicated volume of each sample into 7 wells of the gel in the following order:



Lane 1: M, DNA size marker, 10 µl
Lane 2: CS, green, 20 µl
Lane 3: S1, blue, 20 µl
Lane 4: S2, orange, 20 µl
Lane 5: S3, violet, 20 µl
Lane 6: S4, red, 20 µl
Lane 7: S5, yellow, 20 µl

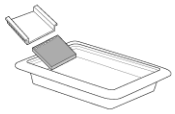
8. Carefully place the lid on the electrophoresis chamber. The lid will attach to the base in only one orientation. The red and black jacks on the lid will match with the red and black jacks on the base. Plug the electrodes into the power supply, red to red and black to black.



9. Turn on the power and electrophorese your samples at 100 V for 30 minutes.

Visualization of DNA Fragments

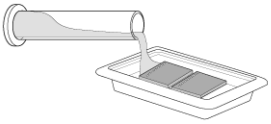
1. When the electrophoresis run is complete, turn off the power and remove the top of the chamber. Carefully remove the gel and tray from the gel box. Be careful — the gel is very slippery. Slide the gel into the staining tray.



2. You have two options for staining your gel:

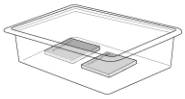
Quick staining (requires 12–15 minutes)

a. Add 120 ml of 100x Fast Blast stain into a staining tray (2 gels per tray).



b. Stain the gels for 2 minutes with gentle agitation. Save the used stain for future use.

c. Transfer the gels into a large washing container and rinse with warm (40–55°C) tap water for approximately 10 seconds.

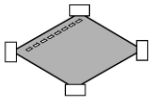


d. Destain by washing twice in warm tap water for 5 minutes each with gentle shaking for best results.

e. Record results.

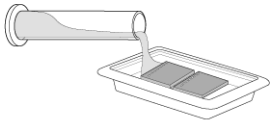
f. Trim away any unloaded lanes.

g. Air-dry the gel on gel support film and tape the dried gel into your laboratory notebook.



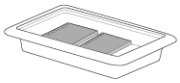
Overnight staining

a. Add 120 ml of 1x Fast Blast DNA stain to the staining tray (2 gels per tray).



b. Let the gels stain overnight, with gentle shaking for best results. No destaining is required.

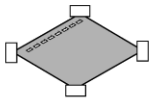
c. Pour off the water into a waste beaker.



d. Record results.

e. Trim away any unloaded lanes.

f. Air-dry the gel on gel support film and tape the dried gel into your laboratory notebook.



Get A Clue Equipment Needed for Wet-lab						
Vendor	**Catalog Number**	**Item**	**Unit**	**Price**	**Minimum Purchase**	**TOTAL**
REQUIRED EQUIPMENT						
For Students' Workstations						
Bio-Rad	166-0007EDU	Forensic DNA Fingerprinting Kit	1	$95.00	1	$95.00
Bio-Rad	164-5050EDU	PowerPac Basic Power Supply	1	$325.00	2	$650.00
Bio-Rad	166-4000EDU	Mini-Sub GT Cell Tank and Lid	1	$169.00	8	$1,352.00
Bio-Rad	166-0506EDU	2-20 µl digital micropipet	1	$159.00	8	$1,272.00
Bio-Rad	223-9040EDU	100-1,000 µl pipet tips BR-40 tips	1 bag of 500	$12.00	1	$12.00
Bio-Rad	223-9035EDU	2-200 µl pipet tips BR-35 tips	1 bag of 1,000	$24.00	1	$24.00
For Instructor's Workstation						
Bio-Rad	166-0507EDU	Adjustable 20-200 µl micropipet	1	$159.00	1	$159.00
Bio-Rad	166-0508EDU	Adjustable 100-1,000 µl micropipet	1	$159.00	1	$159.00
					EQUIPMENT TOTAL	**$3,723.00**
OPTIONAL EQUIPMENT						
Bio-Rad	166-0504EDU	Water bath	1	$505.00		
Bio-Rad	166-0603EDU	Mini centrifuge	1	$260.00		
Bio-Rad	166-0709EDU	Rocking platform	1	$575.00		
Bio-Rad	170-2984EDU	Gel support film (50 sheets)	1 pack	$78.00		
		Microwave oven	1			
OPTIONAL CONSUMABLES & REFILLS						
Bio-Rad	166-0027EDU	DNA Fingerprinting Kit Refill Package (contains crime scene and suspect DNA samples, EcoRI/Pstl restriction enzyme mix, sample loading buffer, Fast Blast DNA stain, DNA size standard, sterile water)	1	$79.00		

GET A CLUE IMPLEMENTATION PLAN — POST-LAB			
Activity	Estimated Time	Materials/Equipment	Purpose/Objectives/ Essential Question
Use Inquiry methods to encourage students to analyze and discuss their electrophoresis results. Sample Questions: • What do your results indicate? • Were your results the same as your classmates? Why or why not? • Explain how the DNA moves through the gel. • How might your results have been improved?	15 minutes	Polaroid pictures of the students' gels from the previous day Teachers Guide: Wet Lab Possible explanations for poor banding patterns	**Purpose:** To explain the results of gel electrophoresis using DNA collected from a hypothetical crime scene and two suspects. **Objectives:** • To build confidence in the students' ability to do science. • To communicate, in written or verbal form, the results of electrophoresis. **Essential Question:** Based on the results of DNA electrophoresis, who is guilty of the crime?
Get a Clue Quiz Game	20 minutes	Question and answer sheets provided; electronic version of game provided on CD	
The Mock Trial Students take on the roles of trial participants. The class conducts a trial complete with videotaping or written reports.	50 minutes	Access to Internet in order to research roles: judge, jury, prosecuting attorney, defense attorney, members of the media, defendant, and bailiff	
Video: "The Footpath Murders" and Discussion.	40 minutes	Films for the Humanities and Science	
You may also select an option from the "Additional Activities" section of your notebook. A variety of activities are included to meet the varying needs and learning styles of your students.			

Alignment with NC Competency Goals	
Biology	
Goal 1 Objectives 1.01, 1.02, 1.03, 1.05 Goal 2 Objectives 2.01, 2.02, 2.04	Goal 3 Objectives 3.01, 3.04

Get A Clue Quiz Game Questions

	Genetic Code	DNA and Such	Get A Clue	Gel Electrophoresis	DNA Dictionary	Hodge Podge
200	Fact or Fiction: 99.9% of a person's DNA is identical to everyone else's DNA.	Fact or Fiction: All twins have identical DNA.	Fact or Fiction: The process used in the wet lab is called electrolysis.	Fill in the blank: In the wet lab, we used gel electrophoresis to obtain a _____ from blood found at a crime scene as well as two suspects.	Define nucleotides.	How many friction stops were on the plunger of the micropipet used in the wet lab?
400	What is DNA's electrical charge?	What is the symbol for microliter, and how many microliters would equal 1 ml?	Fill in the blank: Gel electrophoresis was used in the wet lab to sort DNA by_____.	When using gel electrophoresis to get a DNA fingerprint, it is important that the wells of the gel are located at which end of the chamber?	What is the term for a sequence of DNA that codes for a protein and determines a trait?	Convert 2.7 ml to µl.
600	What is a DNA palindrome?	What piece of equipment was used in the wet lab to load DNA samples into the wells of the gel?	What force was used to move the DNA fragments through the sieve?	What clue can you use to be sure that electricity is properly being conducted through your electrophoresis chamber?	Define restriction enzymes.	In the DNA double helix, what type of bonds holds a nucleotide on one strand of DNA to its complement nucleotide on the other strand?
800	What are the four nucleotides that make up DNA, and which nucleotides pair up?	Give the nucleotide sequence that would complement this strand of DNA: CTTGACTTGGACC.	Which suspect cannot be excluded using the gel electrophoresis results?	Why is it important to fill an electrophoresis chamber with buffer when running an experiment? Give 2 reasons.	What is polymerase chain reaction (PCR)?	What mRNA sequence would be created from the following DNA strand: CTTGACTTGGACC?
1000	What does DNA stand for, and why is it important?	Aside from being used in crime scene investigations, name two other ways that DNA fingerprinting can be useful.	When the gel electrophoresis was finished, why was there a series of blue bands on the agarose gel?	Why is it important that samples loaded into wells during electrophoresis contain loading dye (lead dye)?	What is the human genome?	How does DNA code for a specific trait such as hair color?

Get A Clue Quiz Game Answers

KEY

	Genetic Code	DNA and Such	Get A Clue	Gel Electro-phoresis	DNA Dictionary	Hodge Podge
200	Fact. It is the 0.1% of our DNA that makes us genetically unique.	Fiction: Only identical twins have identical DNA; fraternal twins do not.	Fiction. The process used in the wet lab is called gel electrophoresis.	DNA fingerprint. DNA banding pattern would also be an acceptable answer.	The four basic units that make up the DNA molecule: adenine (A), cytosine (C), guanine (G), and thymine (T).	2.
400	DNA is negatively charged.	µl is microliter. 1000 µl = 1 ml	Size.	Negative end.	Gene.	2.7 ml = 2700 µl
600	A DNA palindrome is a location on a double strand of DNA whose 5' to 3' base pair sequence is identical on each strand.	Digital micropipet. Micropipet also would be an acceptable answer.	Electricity.	Looking at the negative end of the chamber, you should see tiny bubbles forming.	Enzymes that act as "molecular scissors" to cut the DNA at specific sequences of nucleotides or base pairs.	Hydrogen bond.
800	Adenine, guanine, cytosine, thymine. Adenine pairs with thymine; guanine pairs with cytosine.	The nucleotide sequence of the complement strand would read: GAACTGAACCTGG	Any suspect whose DNA fingerprint matches the crime-scene DNA fingerprint cannot be excluded.	Buffer stabilizes the pH, preventing DNA from breaking down. It also conducts electricity.	PCR is a method used to make multiple copies of DNA.	The mRNA sequence would read: GAACUGAACCUGG
1000	Deoxyribonucleic acid. DNA is important because it acts as the blueprint for all living organisms. In humans, DNA codes for everything that we are genetically, for instance, how tall we will be, what color hair and eyes we will have, etc.	DNA fingerprinting can be used as a paternity test for children, and as a way to genetically track a person's ancestry.	The DNA had been cut by a restriction enzyme into many different size pieces. When it was placed in the wells and the electricity was turned on, the negatively charged DNA was attracted to the positive end of the chamber and moved through the gel. DNA fragments moved different distances according to size (smallest fragments moved farthest), and the result was a distinct banding pattern.	The dye runs ahead of the DNA, allowing us to prevent running the gels for too long. If the gels were run too long, the DNA would migrate off the end of the gel and no DNA fingerprint would be obtained.	The human genome is the complete DNA sequence of humans.	DNA is used to create mRNA, which codes for a specific combination of amino acids. Those amino acids form proteins. Proteins act by themselves or in combination with other proteins to give an organism a trait (for instance, hair color).

GET A CLUE IMPLEMENTATION PLAN — ADDITIONAL ACTIVITIES & RESOURCES			
Activity	Estimated Time	Materials/Equipment	Subjects Covered
DNA Restriction Enzyme and Probe Activity Students review the action of restriction enzymes and specific probes.	20 minutes	Photocopies of the worksheet for each student	DNA Medical diagnostics
Taking Fingerprints Activity Students gain an understanding of the techniques used in gathering and analyzing fingerprints	50 minutes	Pencils, a roll of ¾ inch clear tape and a magnifying hand lens	Forensics
Blood Typing: Practice Using Punnett Squares	20 minutes	Photocopies of handout for each student	Genetics
A Paternity Case This activity simulates a paternity case. Students analyze DNA typing data to determine whether I.M. Megabucks, a recently deceased megabillionaire, is actually the father of any of the individuals alleged to be his heir. Source: *Recombinant DNA and Biotechnology — A Guide for Teachers by Kreuzer and Massey*	50 minutes	Photocopies of the student activity pages	DNA fingerprinting Forensics
A Crime on Campus Students become forensic scientists as they compare DNA models of semen taken from a rape victim to models of DNA from three suspects.	30 minutes	Instruction sheets	Forensics
The Ima Mystery Case A forensic anthropologist looks at DNA evidence to establish the identity of human remains.	30 minutes	Instructions Models of DNA taken from an archaeological site and four other locations, any of which may or may not be geneticially connected.	Social studies Forensics
DNA Goes to the Races This is a reading and paper activity that introduces electrophoresis. Source: *Recombinant DNA and Biotechnology — A Guide for Teachers* by Kreuzer and Massey	50 minutes	Photocopies of: DNA Goes to the Races student reading pages, restriction maps, and gel outline	Critical reading
Additional Resources: Groleau, Rick (2000, November). Create a DNA Fingerprint. Retrieved August 11, 2006, from NOVA Online Web site: http://www.pbs.org/wgbh/nova/sheppard/analyze.html (2002). DNA From the Beginning. Retrieved August 11, 2006, from Dolan DNA Learning Center Web site: http://www.dnaftb.org/dnaftb/			

NAME _____

DNA RESTRICTION ENZYME AND PROBE WORKSHEET

Use this strand of DNA to answer the questions that follow:

5' ATTGGATCCCTGAGATCCGATGGATCCGGATCTT 3'
3' TAACCTAGGGACTCTAGGCTACCTAGGCCTAGAA 5'

1. The restriction enzyme BamHI cleaves (cuts) double-stranded DNA at this sequence: 5'\GGATCC\3'.
3'\CCTAGG\5'

It makes a blunt cut each time it finds this sequence in the fragment. Indicate where each cut would occur above by drawing a block to surround the selected sequence.

A variety of health problems result from slight variations in gene sequences, some of which can be inherited. Using DNA probes, scientist can identify some of the unique genetic sequences that are associated with particular diseases. To perform this kind of genetic diagnostic test, scientists create a single strand of DNA to be used as a probe. A signal is attached to the probe so it will glow, change color, or give off detectable radioactivity. If the probe finds a complementary match in the DNA sample, the probe will stick to it and the sample will give off a signal.

2. Would this probe find a complementary sequence to the 5' to 3' strand?

A. 3' C C T A G 5' yes or no

B. 3' A G A T C C 5' yes or no

3. Which of these probes would be better for picking and determining one specific piece of DNA from a mixture of pieces? Why?

A. 3' A-T-G 5'

B. 3' A T G G G T A C T G G G 5'

4. Which of the probes in question 3 would migrate farther on an electrophoresis gel? Why?

5. Explain the diagram below using the terms "restriction enzyme," "restriction site," and "RFLP."

MstII MstII MstII

Normal beta-globin gene

CCTAGG CCTAGG CCTGAGG

Fragment_____ _____

1,150 base pairs 200 bBase pairs

MstII MstII

Beta-globin gene with sickle cell mutation

CCTGAGG CCTGUGG

CCTGAGG Fragment

_____

1,350 base Pairs

NAME _____

KEY

DNA RESTRICTION ENZYME AND PROBE WORKSHEET

Use this strand of DNA to answer the questions that follow:

5' ATTGGATCCCTGAGATCCGATGGATCCGGATCTT 3'
3' TAACCTAGGGACTCTAGGCTACCTAGGCCTAGAA 5'

1. The restriction enzyme BamHI cleaves (cuts) double-stranded DNA at this sequence: 5'\GGATCC\3'.
3'\CCTAGG\5'

It makes a blunt cut each time it finds this sequence in the fragment. Indicate where each cut would occur above by drawing a block to surround the selected sequence.

2. Would this probe find a complementary sequence to the 5' to 3' strand?

A. 3' C C T A G 5' yes or no

B. 3' A G A T C C 5' yes or no

3. Which of these probes would be better for picking and determining one specific piece of DNA from a mixture of pieces? Why?

A. 3' A-T-G 5'

B. 3' A T G G G T A C T G G G 5'

Probe B. Because it is more discriminating (less general).

4. Which of the probes in question 3 would migrate farther on an electrophoresis gel? Why?

Probe A. Because it is smaller.

5. Explain the diagram below using the terms "restriction enzyme," "restriction site," and "RFLP."

MstII MstII MstII
Normal beta-globin gene
CCTAGG CCTAGG CCTGAGG

Fragment_____ _____
1,150 base pairs 200 base pairs

MstII MstII
Beta-globin gene with sickle cell mutation
CCTGAGG CCTGUGG
CCTGAGG Fragment
1,350 base Pairs

MstII is a restriction enzyme that cuts DNA at specific nucleotide sequences called restriction sites. The fragments that result from the cut vary in length. These variations (polymorphisms) can mark the presence or absence of identified genes. When fragments are separated and identified, they are often call RFLPs (restriction fragment length polymorphisms).

NAME _____

THE IMA MYSTERY CASE

You are a forensic anthropologist who recently uncovered an unmarked grave during an archaeological excavation. Circumstantial evidence suggests that these are the remains of Ima Mystery, an early settler on the coast of North Carolina. Ima Mystery's journal, discovered at a yard sale in Wilmington in 1965, has been the source of many interesting, though perhaps fictional, details about life in the Carolinas in the 17th century.

You will compare the DNA of the remains found at the archaeological site with four additional DNA samples.

- Model the action of restriction enzyme EcoRI, by looking for the sequence "GAATTC". Locate the sequence and draw a line on your paper after the "G" each time you find the sequence in the DNA fragments
- Count the number of base pairs in each fragment and place that number below in the chart provided.
- You can eliminate evidence when the DNA fragments do not match the sample you found in the unmarked grave.

DNA SAMPLE FOUND AT THE ARCHEOLOGICAL SITE
5' GGGGGAATTCACGAGAATTC 3'

DNA FROM EXHUMED BODY OF ENGLISHMAN LORD JOHN KIDDING, WHO IMA CLAIMED WAS HER FATHER
5' GGGATTCATACGAATTCCCC 3'

DNA FROM LOCK OF HAIR FEATURED IN THE MUSEUM EXHIBIT "IMA MYSTERY: FACT OR FICTION?"
5' GGAATTCCATACGAGTTCCC 3'

DNA FROM MISSY MYSTERY, WHO CLAIMS TO BE A DESCENDENT OF IMA MYSTERY
5' GGGGGAATTCACGAGAATTC 3'

DNA FROM MRS. JANE MYSTERY-SMITH, WHO ALSO CLAIMS TO BE A DESCENDENT OF IMA MYSTERY
5' GGGAATTCATACGAATTCCC 3'

ANALYSIS OF RESULTS

	DNA From Site	DNA From Lord John	DNA From Lock of Hair	DNA From Missy Mystery	DNA From Jane Mystery-Smith
Number of fragments					
Size of Fragments					

Explain these results.

NAME _____

KEY

THE IMA MYSTERY CASE

You are a forensic anthropologist who recently discovered an unmarked grave during an archaeological excavation. Circumstantial evidence suggests that these are the remains of Ima Mystery, an early settler on the coast of North Carolina. Ima Mystery's journal, discovered at a yard sale in Wilmington in 1965, has been the source of many interesting, though perhaps fictional, details about life in the Carolinas in the 17th century.

You will compare the DNA of the remains found at the archaeological site with four additional DNA samples.

- Model the action of restriction enzyme EcoRI, by looking for the sequence "GAATTC". Locate the sequence and draw a line on your paper after the "G" each time you find the sequence in the DNA fragments
- Count the number of base pairs in each fragment and place that number below in the chart provided.
- You can eliminate evidence when the DNA fragments do not match the sample you found in the unmarked grave.

DNA SAMPLE FOUND AT THE ARCHEOLOGICAL SITE

5' GGGGG|AATTCACGAG|AATTC 3'
5 10 5

DNA FROM EXHUMED BODY OF ENGLISHMAN LORD JOHN KIDDING, WHO IMA CLAIMED WAS HER FATHER

5' GGGATTCATACG|AATTCCCC 3'
12 8

DNA FROM LOCK OF HAIR FEATURED IN THE MUSEUM EXHIBIT "IMA MYSTERY: FACT OR FICTION?"

5' GG|AATTCCATACGAGTTCCC 3'
2 18

DNA FROM MISSY MYSTERY, WHO CLAIMS TO BE A DESCENDENT OF IMA MYSTERY

5' GGGGG|AATTCACGAG|AATTC 3'
5 10 5

DNA FROM MRS. JANE MYSTERY-SMITH, WHO ALSO CLAIMS TO BE A DESCENDENT OF IMA MYSTERY

5' GGG|AATTCATACG|AATTCCC 3'
3 10 7

ANALYSIS OF RESULTS

	DNA From Site	DNA From Lord John	DNA From Lock of Hair	DNA From Missy Mystery	DNA From Jane Mystery-Smith
Number of fragments	3	2	2	3	3
Size of Fragments	5, 10, 5	12, 8	2, 18	5, 10, 5	3, 10, 7

Explain these results.

The DNA from Missy Mystery matches the DNA sample taken from the archaeological site.

NAME _____

TAKING FINGERPRINTS ACTIVITY

OBJECTIVES

Student will gain an understanding of the technique for gathering fingerprints.

MATERIALS NEEDED (for a group of 4 students)

- 4 pencils
- One roll of ¾-inch clear tape
- 4 magnifying hand lens

PROCEDURE

- Each student is responsible for making his or her own fingerprints and will do so with all five fingers on one hand only.

1. Using the following Graphite Table, rub your pencil in each space until there is a sufficient amount of graphite smudge left in the space. You will need to do this in ALL five different locations of the Graphite Table in order for each of your five fingers to be fingerprinted.

GRAPHITE TABLE

Little Finger	Ring Finger	Middle Finger	Index Finger	Thumb

2. Begin your fingerprints with the little finger. Rub your finger on Little Finger smudge mark until the fingertip is completely covered with graphite.

3. Remove a piece of tape approximately 3 inches long and place it over the entire fingertip. Make sure to press down the tape GENTLY on the fingertip.

4. Carefully remove the tape from the fingertip and stick it in the Little Finger space on the following Fingerprint Recording Table.

FINGERPRINT RECORDING TABLE

Little Finger	Ring Finger	Middle Finger	Index Finger	Thumb

• Repeat steps 2, 3, and 4 for each of the remaining fingers.

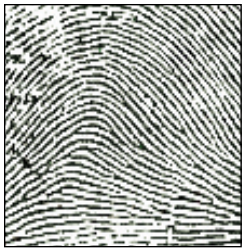
5. Upon completion of all 5 fingerprints, carefully examine each with the magnifying hand lens. Try to identify the types of fingerprints you have by using the following Fingerprint Types chart:

LOOP

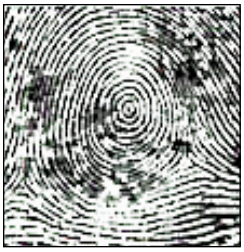
DOUBLE LOOP

CENTRAL POCKET LOOP

TENTED ARCH



PLAIN ARCH



PLAIN WHORL

ACCIDENTAL

6. In the following Fingerprints Data Table, record the types of fingerprints you and the other members of your group have.

FINGERPRINTS DATA TABLE

Student	Little Finger	Ring F inger	Middle Finger	Index Finger	Thumb

7. Once everyone has finished recording their fingerprints, have all groups report the results to the rest of the class. Each student should make tally marks in the Class Fingerprints Data Table for the entire class's results.

CLASS FINGERPRINTS DATA TABLE

Loop	Double Loop	Central Pocket Loop	Tented Arch	Plain Arch	Plain Whorl	Accidental

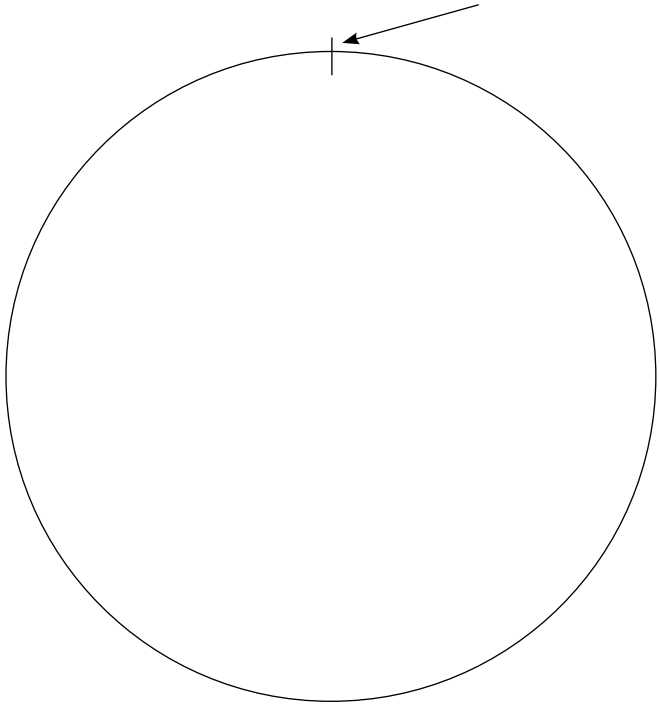
8. Upon completion of the class results, each student will use the "Total" amounts in the above table to calculate (a) the percentage of classmates with that particular fingerprint type and (b) the degrees of a pie graph that percentage represents. Record your results in the following Fingerprint Type Percentage/Degrees Data Table.

- To calculate the percentage, take the total number of students with that particular fingerprint type (from the table in step 7) and divide by the total number of students in the class. For example, if 12 students in a class of 28 have one particular fingerprint type, it will look like this: 12/28 = .4285. Round your value to the nearest 100th, and the answer will be 0.43, which equals 43%. Show all math in the space provided.
- To calculate the degrees of a pie graph and using the above example, take .43 and multiply it by 360. For example: 0.43 x 360 = 154.8.

FINGERPRINT TYPE PERCENTAGE/DEGREES DATA TABLE

Loop	Double Loop	Central Pocket Loop	Tented Arch	Plain Arch	Plain Whorl	Accidental
% =	% =	% =	% =	% =	% =	% =
Degrees =	Degrees =	Degrees =	Degrees =	Degrees =	Degrees =	Degrees =

9. Once the percentage and degrees values are calculated, each student will need to create a pie graph illustrating the types of fingerprints represented by all class members. In the circle provided, use a protractor to mark off the calculated values. As if you were looking at a clock, begin at the 12 o'clock mark.



10. Is one type of fingerprint more common than another type? _____

11. Is one type relatively rare? _____

NOTE: An Extension Activity could be independent research on the fingerprints of relatives in a student's family. After collecting the data and making the appropriate calculations, students should be able to hypothesize whether or not fingerprints are inherited.

NAME _____

BLOOD TYPING: PRACTICE USING PUNNETT SQUARES

OBJECTIVES

Students will use their knowledge of genetics and blood typing to practice Punnett squares in determining the probability of possible offspring from particular parents.

BACKGROUND INFORMATION

A genetic cross can be used to determine the possible types of offspring from parents with a specific blood type. The Punnett square is the tool used to predict the genetic variations that can result from such a cross. Individuals with two identical alleles for a blood type are known as homozygotes. Those individuals with two different alleles for a blood type are known as heterozygotes.

The likelihood of a particular event happening is known as probability. Probabilities can predict the average outcome of a test cross, but they will not give an exact answer for the expected outcome.

	X	Y
X	XX	XY
X	XX	XY

XX = Homozygotes
XY = Heterozygotes

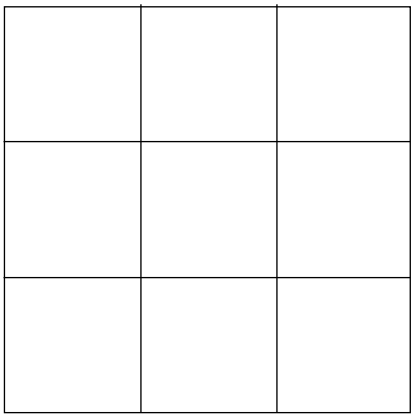
ABO blood groups in humans are an example of multiple alleles of a single gene. A, B, AB, and O are the four possible blood type phenotypes seen in humans. These blood groups will result from many different combinations of the three different alleles for one gene (symbolized as I^A, I^B, and i) could result in six possible genotypes.

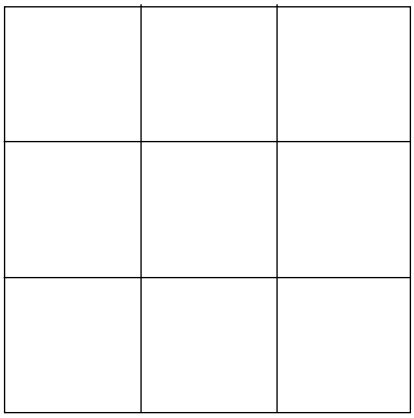
Blood type A will have I^AI^A and I^Ai; blood type B will have I^BI^B and I^Bi; blood type AB will have I^AI^B; and blood type O will have *ii*. **Both blood types A and B have homozygous and heterozygous combinations; blood type AB has only a heterozygous combination; and blood type O has only a homozygous combination.** In the ABO blood typing system, the *A* and *B* alleles are codominant, and both are dominant to the *O* allele.

PROCEDURE

Your assignment is to complete the Punnett square to determine the probability of the following paternity dispute. A woman with type AB blood claims that a man with type A blood is the father of her type O child. Using the evidence given, is it possible that this man actually fathered her child?

NOTE: You will need to fill in the box with the possible alleles, as well as with the possible offspring this couple could produce. Remember that type A blood can have two different genotypes. You will need to complete both Punnett squares to be able to solve this problem. Probability is expressed in a percentage value.





RESULTS & ANALYSIS

1. Using the Punnett squares, is it possible for a woman with blood type AB and a man with blood type A to produce a child with type O blood?

2. If the father is homozygous for blood type A, what is the probability that offspring produced with the type AB mother will have blood type:

a. homozygous A _____ c. heterozygous A _____

b. homozygous B _____ d. heterozygous B _____

3. If the father is heterozygous for blood type A, what is the probability that offspring produced with the same blood type AB mother will have blood type:

a. homozygous A _____ c. heterozygous A _____

b. homozygous B _____ d. heterozygous B _____

NAME _____

KEY

BLOOD TYPING: PRACTICE USING PUNNETT SQUARES

PROCEDURE

Your assignment is to complete the Punnett square to determine the probability of the following paternity dispute. A woman with type AB blood claims that a man with type A blood is the father of her type O child. Using the evidence given, is it possible that this man actually fathered her child?

NOTE: You will need to fill in the box with the possible alleles, as well as with the possible offspring this couple could produce. Remember that type A blood can have two different genotypes. You will need to complete both Punnett squares to be able to solve this problem. Probability is expressed in a percentage value.

	I^A	I^B
I^A	$I^A I^A$	$I^A I^B$
I^A	$I^A I^A$	$I^A I^B$

	I^A	I^B
I^A	$I^A I^A$	$I^A I^B$
I^i	$I^A I^i$	$I^B I^i$

RESULTS & ANALYSIS

1. Using the Punnett squares, is it possible for a woman with blood type AB and a man with blood type A to produce a child with type O blood?

No.

2. If the father is homozygous for blood type A, what is the probability that offspring produced with the type AB mother will have blood type:

a. homozygous A 50%

b. homozygous B 50%

c. heterozygous A 0%

d. heterozygous B 0%

3. If the father is heterozygous for blood type A, what is the probability that offspring produced with the same blood type AB mother will have blood type:

a. homozygous A 25%

b. homozygous B 25%

c. heterozygous A 25%

d. heterozygous B 25%

A CRIME ON CAMPUS

At 12:30 a.m. on April 24, 2006, while returning to her dormitory after a study session at a friend's apartment, a college student was attacked.. A campus security guard discovered the young woman in a state of shock and accompanied her to the hospital, where she received medical assistance. A nurse collected samples of semen from the woman's body, and some of her stained clothing was also saved as physical evidence. Based on the young woman's description of her attacker, three men were identified as suspects.

Each of you will assist in this case as forensic scientist. You will be presented with models of the four DNA samples (semen from the woman, suspect #1, suspect #2, and suspect #3. Your assignment will be to analyze the four samples.

1. Use the restriction enzyme EcoRI to cut all four samples into RFLPs on the DNA Fragment For Analysis Sheet. At the bottom of the DNA Fragment For Analysis for Sheet, be sure to circle EcoR1 and its recognition site.

2. Count the number of base pairs in each fragment. Write the number of bases you counted above each fragment.

3. On the second DNA Fragment for Analysis Sheet, repeat steps 1 and 2 using the restriction enzyme BamHI.

4. Using the Gel Electrophoresis Lab Report Form, imagine your paper is a gel with wells near the top of the page to place the DNA samples at the negative poles.

5. Assume that the bottom of the page is the most positive end of your gel, and that an electric current has been applied to the gel. The numbers on the left side of your paper represent the size of the RFLPs based on the number of base pairs present in each fragment. Use the information you collected on your DNA Fragment for Analysis Sheet for EcoRI to draw your gel.

6. Repeat steps 4 and 5 on a separate Gel Electrophoresis Lab Report Sheet for the restriction enzyme BamHI.

ENZYME USED: EcoRI

DNA FRAGMENTS FOR ANALYSIS

Victim's Specimen — DNA Sequence

5' TACGAATTCCCTTGGATCCGGCCCTGAATTCAACCTTAGGATCCGAATTCCCCGGTGGATCCCCCGAATTCGGCTGGATCCAGAATTCCCGTCCAGC
3' ATGCTTAAGGGAACCTAGGCCGGGACTTAAGTTGGAATCCTAGGCTTAAGGGGCCACCTAGGGGGCTTAAGCCGACCTAGGTCTTAAGGGCAGGTCG

Suspect 1's Specimen — DNA Sequence

5' TACGAATTCACTGCCTTTGGATCCGGAATTCATGGATCCCAGAGGAATTCCTTGGATCCGGATCCGAATTCAGGGGGATCCCGAATTCAGCAATCCT
3' ATGCTTAAGTGACGGAAACCTAGGCCTTAAGTACCTAGGGTCTCCTTAAGGAACCTAGGCCTAGGCTTAAGTCCCCCTAGGGCTTAAGTCGTTAGGA

Suspect 2's Specimen — DNA Sequence

5' TACGAATTCCCAAGGATCCAAACCCGAATTCAACCGCAGGATCCGAATTCCTAAGGGGATCCCGGGAATTCATTTGGATCCAGAATTCCCCTTAGGC
3' ATGCTTAAGGGTTCCTAGGTTTGGGCTTAAGTTGGCGTCCTAGGCTTAAGGATTCCCCTAGGGCCCTTAAGTAAACCTAGGTCTTAAGGGGAATCCG

Suspect 3's Specimen — DNA Sequence

5' TACGAATTCCCGGGGATCCCTCTGAATTCCAAGAATTCGGATCCGAATTCGAATTCGGATCCCAAAGCCGAATTCGGATCCCGAATTCATCAATTCC
3' ATGCTTAAGGGCCCCTAGGGAGACTTAAGGTTCTTAAGCCTAGGCTTAAGCTTAAGCCTAGGGTTTCGGCTTAAGCCTAGGGCTTAAGTAGTTAAGG

Restriction enzyme recognition site sequence:

EcoRI G|A A T T C
C T T A A|G

ENZYME USED: EcoRI

KEY

DNA FRAGMENTS FOR ANALYSIS

Victim's Specimen — DNA Sequence

```
     4                22                   19                   21                   17                14
5' TACG  AATTCCCTTGGATCCGGCCCTG  AATTCAACCTTAGGATCCG  AATTCCCCGGTGGATCCCCCG  AATTCGGCTGGATCCAG  AATTCCCGTCCAGC
3' ATGCTTAA GGGAACCTAGGCCGGGACTTAA GTTGGAATCCTAGGCTTAA GGGGCCACCTAGGGGGCTTAA GCCGACCTAGGTCTT AAGGGCAGGTCG
```

Suspect 1's Specimen — DNA Sequence

```
     4                22                   19                   21                   17                14
5' TACG  AATTCACTGCCTTTGGATCCGG  AATTCATGGATCCCAGAGG  AATTCCTTGGATCCGGATCCG  AATTCAGGGGGATCCCG  AATTCAGCAATCCT
3' ATGCTTAA GTGACGGAAACCTAGGCCTTAA GTACCTAGGGTCTCCTTAA GGAACCTAGGCCTAGGCTTAA GTCCCCCTAGGGCTTAA GTCGTTAGGA
```

Suspect 2's Specimen — DNA Sequence

```
     4                22                   19                   21                   17                14
5' TACG  AATTCCCAAGGATCCAAACCCG  AATTCAACCGCAGGATCCG  AATTCCTAAGGGGATCCCGGG  AATTCATTTGGATCCAG  AATTCCCCTTAGGC
3' ATGCTTAA GGGTTCCTAGGTTTGGGCTTAA GTTGGCGTCCTAGGCTTAA GGATTCCCCTAGGGCCCTTAA GTAAACCTAGGTCTTAA GGGGAATCCG
```

Suspect 3's Specimen — DNA Sequence

```
     4                20                9               12              6                19                   13                14
5' TACG  AATTCCCGGGGATCCCTCTG  AATTCCAAG  AATTCGGATCCG  AATTCG  AATTCGGATCCCAAAGCCG  AATTCGGATCCCG  AATTCATCAATTCC
3' ATGCTTAA GGGCCCCTAGGGAGACTTAA GGTTCTTAA GCCTAGGCTTAA GCTTAA GCCTAGGGTTTCGGCTTAA GCCTAGGGCTTAA GTAGTTAAGG
```

Restriction enzyme recognition site sequence:

EcoRI G|A A T T C
C T T A A|G

GEL ELECTROPHORESIS LABORATORY REPORT

Type of restriction enzyme used: EcoRI
Recognition site sequence 5' to 3': G|A A T T C
C T T A A|G

	VICTIM	SUSPECT 1	SUSPECT 2	SUSPECT 3
	WELL	WELL	WELL	WELL
30	—	—	—	—
29	—	—	—	—
28	—	—	—	—
27	—	—	—	—
26	—	—	—	—
25	—	—	—	—
24	—	—	—	—
23	—	—	—	—
22	—	—	—	—
21	—	—	—	—
20	—	—	—	—
19	—	—	—	—
18	—	—	—	—
17	—	—	—	—
16	—	—	—	—
15	—	—	—	—
14	—	—	—	—
13	—	—	—	—
12	—	—	—	—
11	—	—	—	—
10	—	—	—	—
9	—	—	—	—
8	—	—	—	—
7	—	—	—	—
6	—	—	—	—
5	—	—	—	—
4	—	—	—	—
3	—	—	—	—
2	—	—	—	—
1	—	—	—	—

Based on the data, who could or could not have left the specimen at the crime scene?

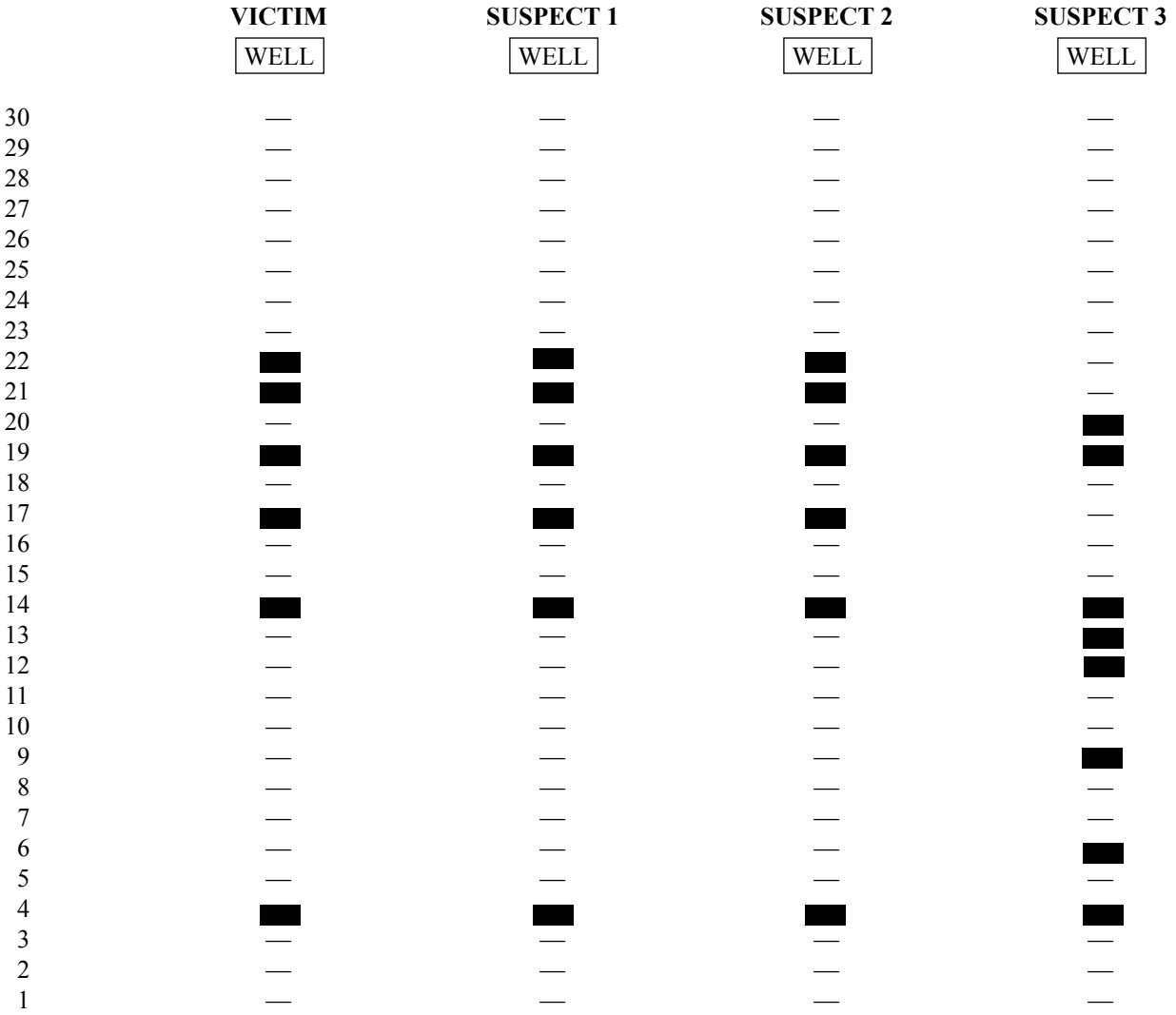
Based on the data, what reason would you give for these conclusions?

Compare your conclusions with those of other student forensic geneticists. Discuss the ethics of using RFLPs as evidence. Should this evidence be used to find someone guilty of a crime? Who has the right to know this information?

GEL ELECTROPHORESIS LABORATORY REPORT

KEY

Type of restriction enzyme used: EcoRI
Recognition site sequence 5' to 3': G|A A T T C
C T T A A|G



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Compare your conclusions with those of other student forensic geneticists. Discuss the ethics of using RFLPs as evidnece. Should this evidence be used to find someone guilty of a crime? Who has the right to know this information?

ENZYME USED: BamHI

DNA FRAGMENTS FOR ANALYSIS

Victim's Specimen — DNA Sequence

5' TACGAATTCCCTTGGATCCGGCCCTGAATTCAACCTTAGGATCCGAATTCCCCGGTGGATCCCCCGAATTCGGCTGGATCCAGAATTCCCGTCCAGC
3' ATGCTTAAGGGAACCTAGGCCGGGACTTAAGTTGGAATCCTAGGCTTAAGGGGCCACCTAGGGGGCTTAAGCCGACCTAGGTCTTAAGGGCAGGTCG

Suspect 1's Specimen — DNA Sequence

5' TACGAATTCACTGCCTTTGGATCCGGAATTCATGGATCCCAGAGGAATTCCTTGGATCCGGATCCGAATTCAGGGGGATCCCGAATTCAGCAATCCT
3' ATGCTTAAGTGACGGAAACCTAGGCCTTAAGTACCTAGGGTCTCCTTAAGGAACCTAGGCCTAGGCTTAAGTCCCCCTAGGGCTTAAGTCGTTAGGA

Suspect 2's Specimen — DNA Sequence

5' TACGAATTCCCAAGGATCCAAACCCGAATTCAACCGCAGGATCCGAATTCCTAAGGGGATCCCGGGAATTCATTTGGATCCAGAATTCCCCTTAGGC
3' ATGCTTAAGGGTTCCTAGGTTTGGGCTTAAGTTGGCGTCCTAGGCTTAAGGATTCCCCTAGGGCCCTTAAGTAAACCTAGGTCTTAAGGGGAATCCG

Suspect 3's Specimen — DNA Sequence

5' TACGAATTCCCGGGGATCCCTCTGAATTCCAAGAATTCGGATCCGAATTCGAATTCGGATCCCAAAGCCGAATTCGGATCCCGAATTCATCAATTCC
3' ATGCTTAAGGGCCCCTAGGGAGACTTAAGGTTCTTAAGCCTAGGCTTAAGCTTAAGCCTAGGGTTTCGGCTTAAGCCTAGGGCTTAAGTAGTTAAGG

Restriction enzyme recognition site sequence:

BamHI G|G A T C C
C C T A G|G

ENZYME USED: BamHI

KEY

DNA FRAGMENTS FOR ANALYSIS

Victim's Specimen — DNA Sequence

	14		25		18		19		21	
5'	TACGAATTCCCTTG		GATCCGGCCCTGAATTCAACCTTAG		GATCCGAATTCCCCGGTG		GATCCCCCGAATTCGGCTG		GATCCAGAATTCCCGTCCAGC	
3'	ATGCTTAAGGGAACCTAG		GCCGGGACTTAAGTTGGAATCCTAG		GCTTAAGGGGCCACCTAG		GGGGCTTAAGCCGACCTAG		GTCTTAAGGGCAGGTCG	

Suspect 1's Specimen — DNA Sequence

	19		15		20	6	16		21	
5'	TACGAATTCACTGCCTTTG		GATCCGGAATTCATG		GATCCCAGAGGAATTCCTTG	GATCCG	GATCCGAATTCAGGGG		GATCCCGAATTCAGCAATCCT	
3'	ATGCTTAAGTGACGGAAACCTAG		GCCTTAAGTACCTAG		GGTCTCCTTAAGGAACCTAG	GCCTAG	GCTTAAGTCCCCCTAG		GGCTTAAGTCGTTAGGA	

Suspect 2's Specimen — DNA Sequence

	14		25		18		19		21	
5'	TACGAATTCCCAAG		GATCCAAACCCGAATTCAACCGCAG		GATCCGAATTCCTAAGGG		GATCCCGGGAATTCATTTG		GATCCAGAATTCCCCTTAGGC	
3'	ATGCTTAAGGGTTCCTAG		GTTTGGGCTTAAGTTGGCGTCCTAG		GCTTAAGGATTCCCCTAG		GGCCCTTAAGTAAACCTAG		GTCTTAAGGGGAATCCG	

Suspect 3's Specimen — DNA Sequence

	14		25		18		19		21	
5'	TACGAATTCCCGGG		GATCCCTCTGAATTCCAAGAATTCG		GATCCGAATTCGAATTCG		GATCCCAAAGCCGAATTCG		GATCCCGAATTCATCAATTCC	
3'	ATGCTTAAGGGCCCCTAG		GGAGACTTAAGGTTCTTAAGCCTAG		GCTTAAGCTTAAGCCTAG		GGTTTCGGCTTAAGCCTAG		GGCTTAAGTAGTTAAGG	

Restriction enzyme recognition site sequence:

BamHI G|GATCC
CCTAG|G

GEL ELECTROPHORESIS LABORATORY REPORT

Type of restriction enzyme used: BamHI
Recognition site sequence 5' to 3': G|G A T C C
C C T A G|G

	VICTIM	SUSPECT 1	SUSPECT 2	SUSPECT 3
	WELL	WELL	WELL	WELL
30	—	—	—	—
29	—	—	—	—
28	—	—	—	—
27	—	—	—	—
26	—	—	—	—
25	—	—	—	—
24	—	—	—	—
23	—	—	—	—
22	—	—	—	—
21	—	—	—	—
20	—	—	—	—
19	—	—	—	—
18	—	—	—	—
17	—	—	—	—
16	—	—	—	—
15	—	—	—	—
14	—	—	—	—
13	—	—	—	—
12	—	—	—	—
11	—	—	—	—
10	—	—	—	—
9	—	—	—	—
8	—	—	—	—
7	—	—	—	—
6	—	—	—	—
5	—	—	—	—
4	—	—	—	—
3	—	—	—	—
2	—	—	—	—
1	—	—	—	—

Based on the data, who could or could not have left the specimen at the crime scene?

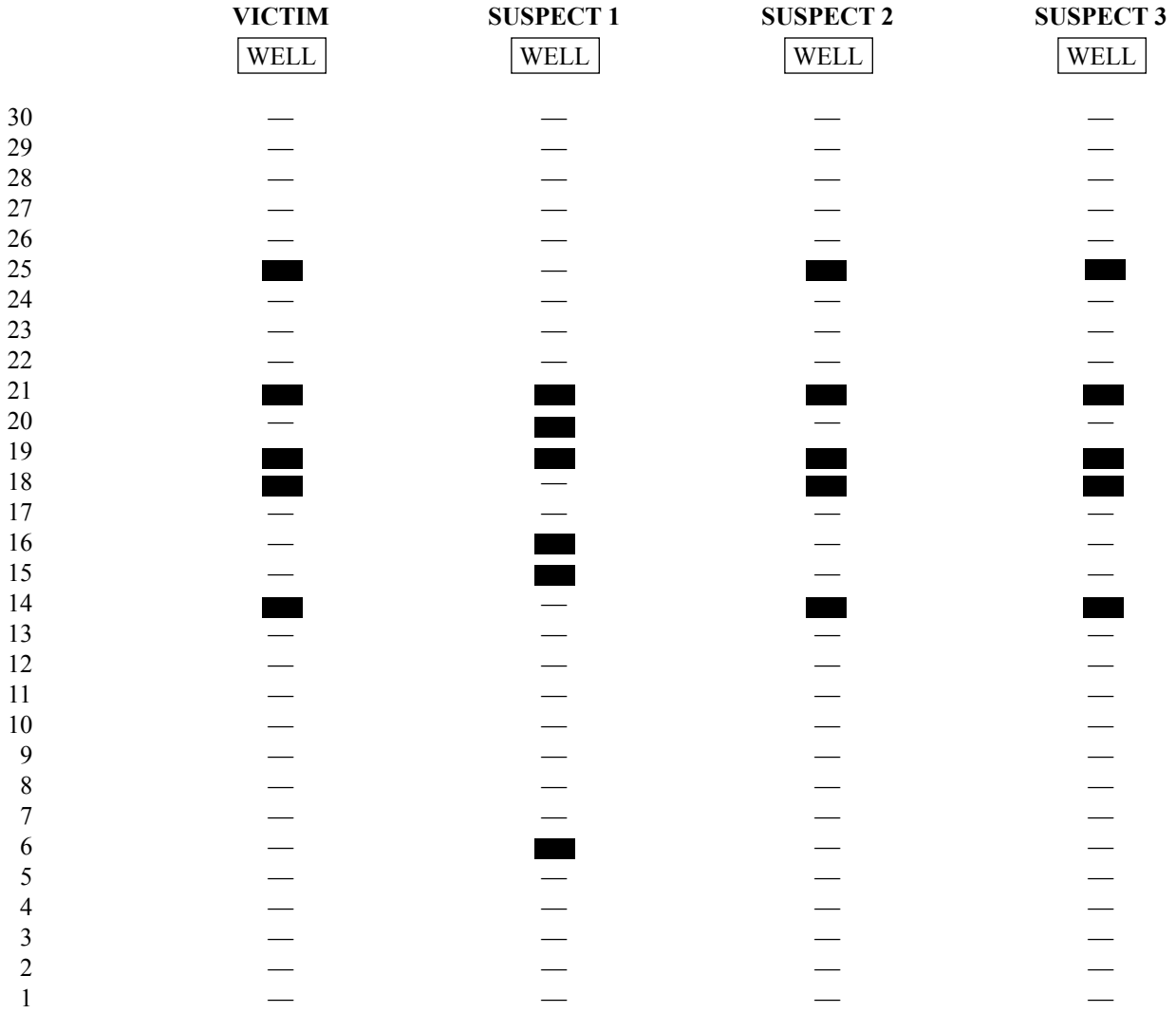
Based on the data, what reason would you give for your conclusions?

Compare your conclusions with those of other student forensic geneticists. Discuss the ethics of using RFLPs as evidence. Should this evidence be used to find someone guilty of a crime? Who has the right to know this information?

GEL ELECTROPHORESIS LABORATORY REPORT

KEY

Type of restriction enzyme used: BamHI
Recognition site sequence 5' to 3': G|A A T T C
C T T A A|G



Based on the data, who could or could not have left the specimen at the crime scene?

Based on the data, what reason would you give for your conclusions?

Compare your conclusions with those of other student forensic geneticists. Discuss the ethics of using RFLPs as evidence. Should this evidence be used to find someone guilty of a crime? Who has the right to know this information?

QUANTITATIVE ANALYSIS OF DNA FRAGMENT SIZES

From Bio-Rad's Forensic DNA Fingerprinting Kit Instruction Manual

If you were on trial, would you want to rely on a technician's eyeball estimate of a match, or would you want some more accurate measurement?

In order to make the most accurate comparison between the crime scene DNA and the suspect DNA, other than just a visual match, a quantitative measurement of the fragment sizes needs to be created. This is done below:

1. Using the ruler, measure the distance (in millimeters) that each of your DNA fragments or bands traveled from the well. Measure the distance from the bottom of the well to the center of each DNA band and record your numbers in the table on the next page. The data in the table will be used to construct a standard curve and to estimate the sizes of the crime scene and suspect restriction fragments.

2. To make an accurate estimate of the fragment sizes for either the crime scene or suspect DNA samples, a standard curve is created using the distance (x-axis) and fragment size (y-axis) data from the known HindIII lambda digest (DNA marker). Using both linear and semilog graph paper, plot distance versus size for bands 2–6. On each graph, use a ruler and draw a line joining the points. Extend the line all the way to the right hand edge of the graph.

Which graph provides the straightest line that you could use to estimate the crime scene or the suspects' fragment sizes? Why do you think one graph is straighter than the other?

3. Decide which graph, linear or semilog, should be used to estimate the DNA fragment sizes of the crime scene and suspects. Justify your selection.

4. To estimate the size of an unknown crime scene or suspect fragment, find the distance that fragment traveled. Locate that distance on the x-axis of your standard graph. From that position on the x-axis, read up to the standard line, and then follow the graph line to over to the y-axis. You might want to draw a light pencil mark from the x-axis up to the standard curve and over to the y-axis showing what you've done. Where the graph line meets the y-axis, this is the approximate size of your unknown DNA fragment. Do this for all crime scene and suspect fragments.

5. Compare the fragment sizes of the suspects and the crime scene. Is there a suspect that matches the crime scene?

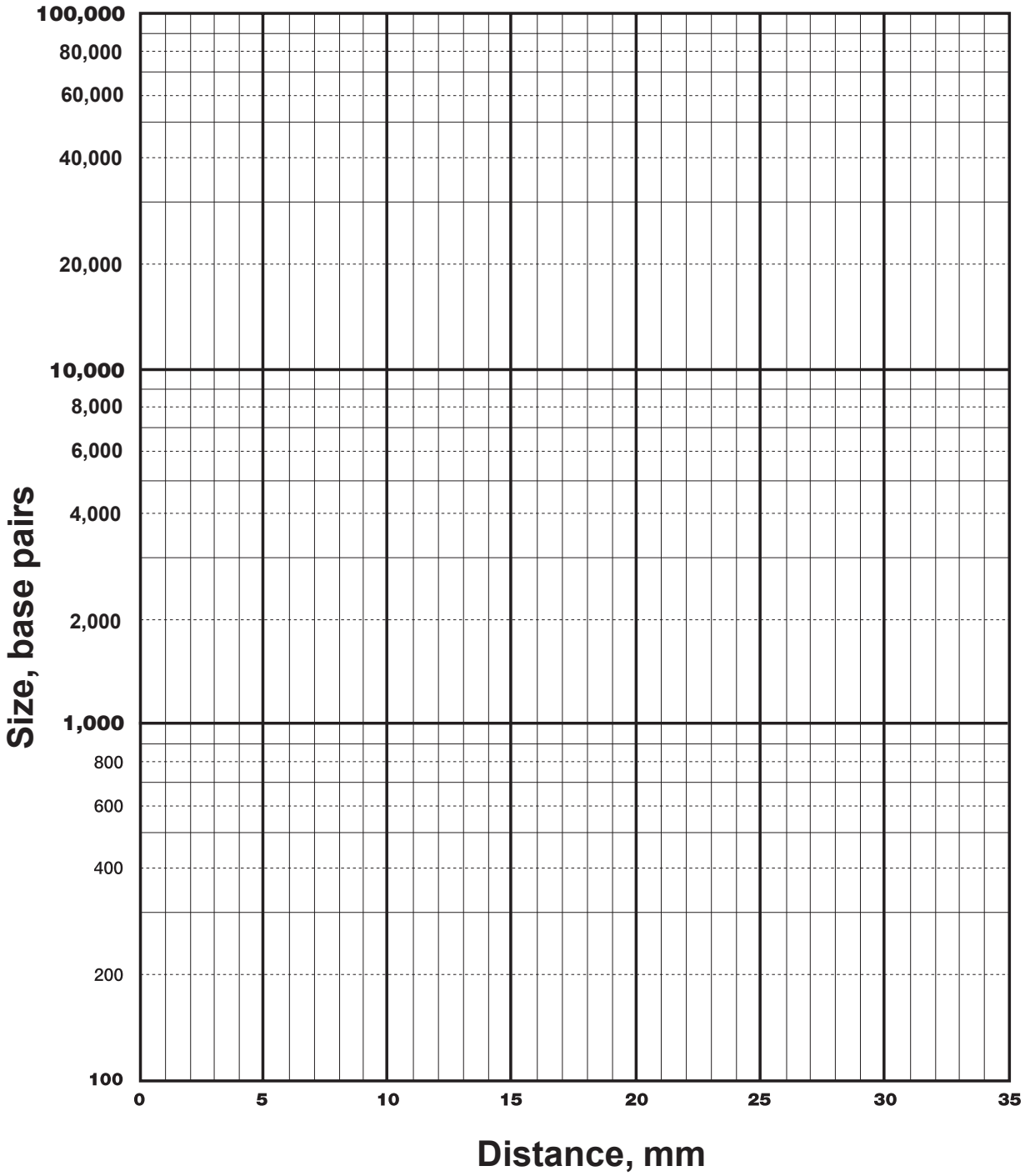
How sure are you that this is a match?

ELECTROPHORESIS DATA

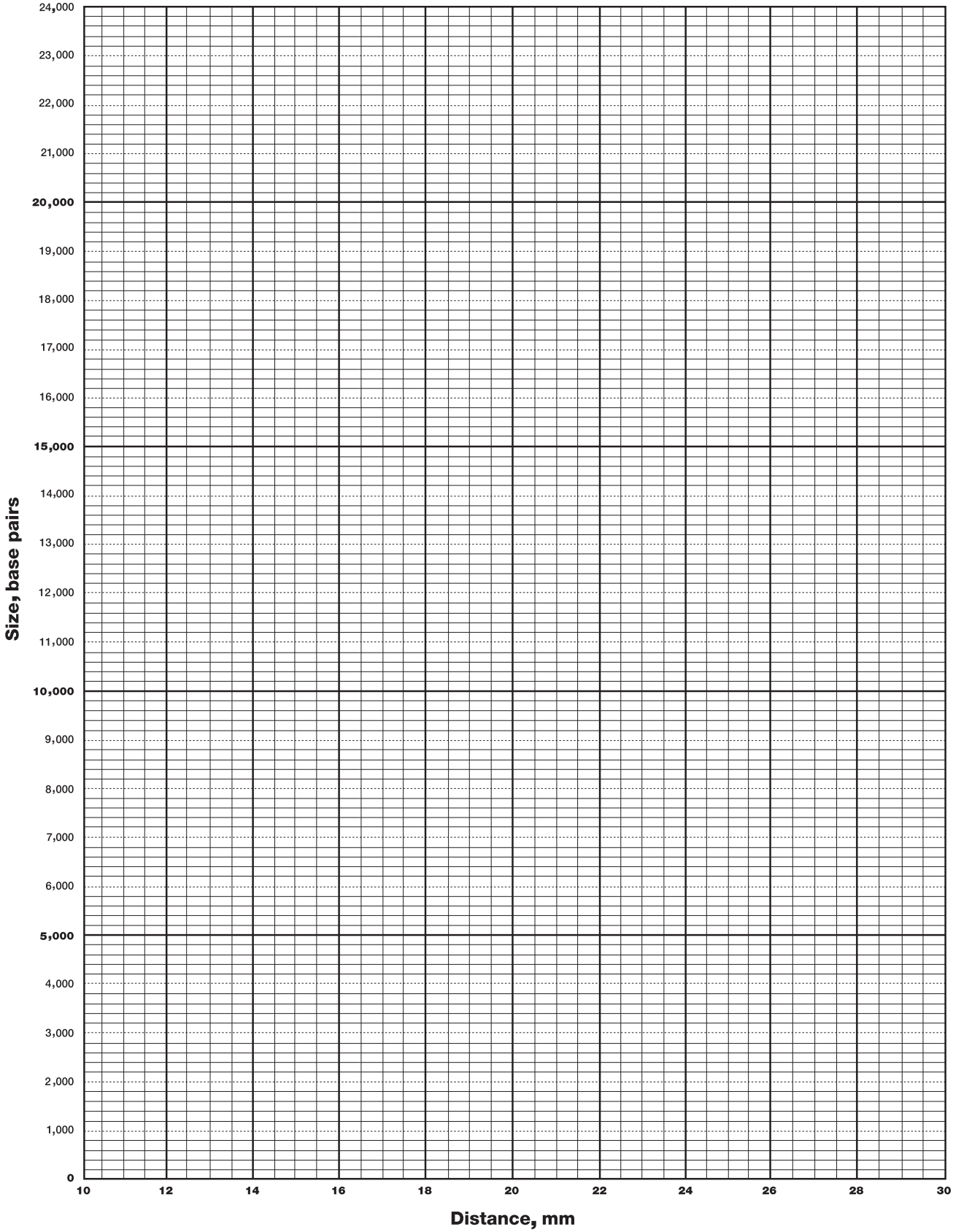
Measure the distance (in millimeters) that each fragment traveled from the well and record it in the table. Estimate its size, in base pairs, by comparing its position to the HindIII lambda DNA markers. Remember that some lanes will have fewer than six fragments.

	Lambda/HindIII Size Marker		**Crime Scene**		**Suspect 1**		**Suspect 2**		**Suspect 3**		**Suspect 4**		**Suspect 5**	
Band	Distance (mm)	Actual Size (bp)	Distance (mm)	Approx. Size (bp)	Distance (mm)	Approx. Size (bp)	Distance (mm)	Approx. Size (bp)	Distance (mm)	Approx. Size (bp)	Distance (mm)	Approx. Size (bp)	Distance (mm)	Approx. Size (bp)
1		23,130												
2		9,416												
3		6,557												
4		4,361												
5		2,322												
6		2,027												

SEMILOG GRAPH PAPER



GRAPH PAPER



INTERPRETATION OF RESULTS

1. What are we trying to determine? Restate the central question.

2. Which of your DNA samples were fragmented? What would your gel look like if the DNA were not fragmented?

3. What caused the DNA to become fragmented?

4. What determines where a restriction enzyme will "cut" a DNA molecule?

5. A restriction enzyme "cuts" two DNA molecules at the same location. What can you assume is identical about the molecules at that location?

6. Do any of your suspect samples appear to have EcoRI or PstI recognition sites at the same location as the DNA from the crime scene?

7. Based on the above analysis, do any of the suspect samples of DNA seem to be from the same individual as the DNA from the crime scene? Describe the scientific evidence that supports your conclusion.

QUANTITATIVE ANALYSIS OF DNA FRAGMENT SIZES

KEY

From Bio-Rad's DNA Fingerprinting Kit Instruction Manual

ELECTROPHORESIS DATA

Measure the distance (in millimeters) that each fragment traveled from the well and record it in the table. Estimate its size (in base pairs) by comparing its position to the HindIII lambda DNA markers. Remember that some lanes will have fewer than six fragments.

	Lambda/HindIII Size Marker		Crime Scene		Suspect 1		Suspect 2		Suspect 3		Suspect 4		Suspect 5***	
Band	Distance (mm)	Actual Size (bp)	Distance (mm)	Approx. Size (bp)	Distance (mm)	Approx. Size (bp)	Distance (mm)	Approx. Size (bp)	Distance (mm)	Approx. Size (bp)	Distance (mm)	Approx. Size (bp)	Distance (mm)	Approx. Size (bp)
1	11.0	23,130	19.0	3,679	21.0	2,860**	21.0	2,860**	19.0	3,679	21.0	2,860**	21.0	2,860**
2	13.0	9,416	20.5	2,860**	23.5	1,199	25.0	1,700	20.5	2,860**	29.5	1,093	24.0	1,986
3	15.0	6,557	32.0	828	30.5	941	28.5	1,159	32.0	828			29.5	1,093
4	18.0	4,361*												
5	23.0	2,322												
6	24.0	2,027												

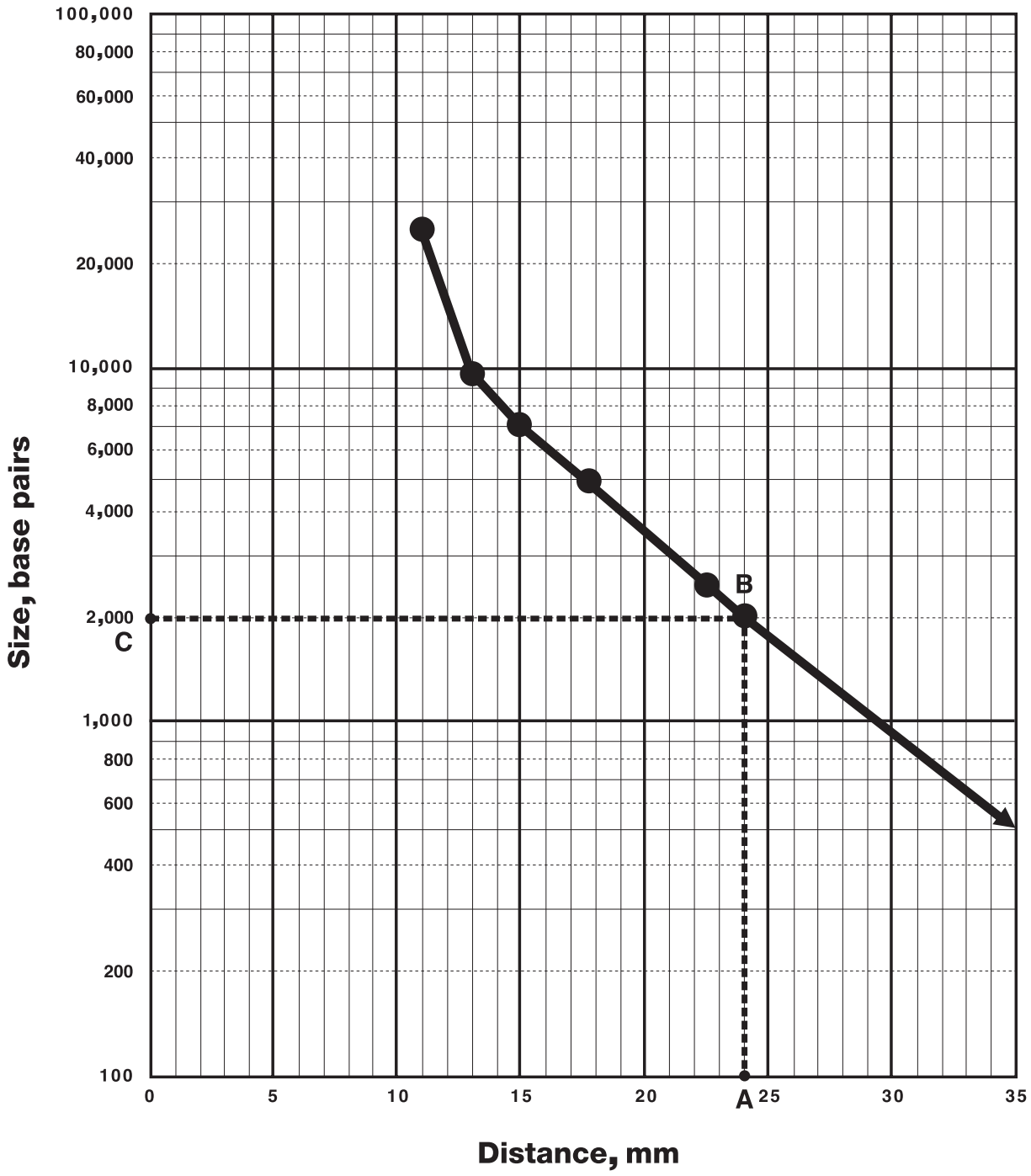
*This fragment may appear faint if the markers were not heated to 65 °C. Lamba HindIII digestion also generates bands of 564 and 125 bp that are usually too faint to see on a gel.

**The measured migration distance for these bands varies depending upon the thickness of the bands.

***S4 and S5 DNA lanes may also contain a very faint band of 500 bp.

DNA STANDARD BAND MIGRATION

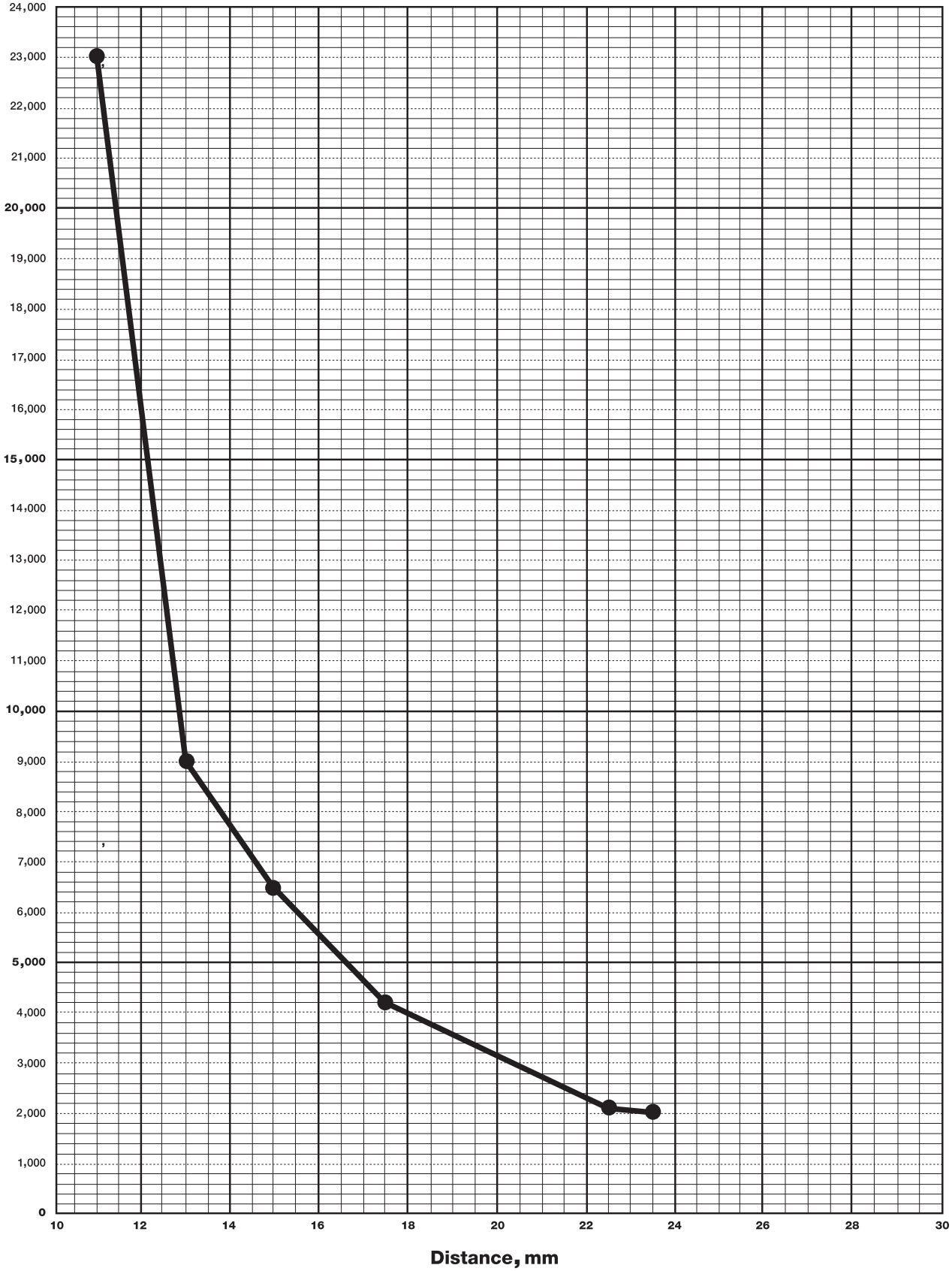
KEY



To estimate the size of any unknown crime scene or suspect fragment, you first need to determine the distances the specific fragment travelled. Locate the distance on the x-axis of your standard graph. For example, suspect 5, band 2 migrated 24 mm (A). From the 24 mm mark on the x-axis, read up to the standard line; when you intersect your standard curve, mark the spot with a shaded circle (B). Follow the intersect point over to the y-axis and determine where the graph line meets the y-axis this is the approximate size of the fragment (C). Suspect 5, band 2 is approximately 2,000 bp. Repeat this procedure for the crime scene and all suspects' fragments. As you determine the approximate fragment sizes, fill in the data in the data table.

KEY

FINGERPRINTING STANDARD CURVE: LINEAR



INTERPRETATION OF RESULTS

KEY

1. What are we trying to determine? Restate the central question.

We are trying to determine if samples of DNA are from the same individual or from different individuals.

2. Which of your DNA samples were fragmented? What would your gel look like if the DNA were not fragmented?

The number of fragmented samples will vary. They will have one band on the gel if the DNA was not cut.

3. What caused the DNA to become fragmented?

The addition of restriction enzymes.

4. What determines where a restriction enzyme will "cut" a DNA molecule?

A special sequence of bases on the DNA called restriction sites.

5. A restriction enzyme "cuts" two DNA molecules at the same location. What can you assume is identical about the molecules at that location?

The restriction sites are identical.

6. Do any of your suspect samples appear to have EcoRI or PstI recognition sites at the same location as the DNA from the crime scene?

The samples in lanes 2 and 5 match (CS and S3).

7. Based on the above analysis, do any of the suspect samples of DNA seem to be from the same individual as the DNA from the crime scene? Describe the scientific evidence that supports your conclusion.

The CS and S3 samples appear to be identical. They both produce similar banding patterns on the gel.

GET A CLUE IMPLEMENTATION PLAN — INTERDISCIPLINARY BRIDGES							
Activity	Arts	English	Health	Math	Science	Social Studies	Provided materials
Mock Trial		X			X	X	Handout with instructions
Integrating Forensics, Civics, and World Literature: The Brothers Karamazov		X			X	X	Instructions
Literary Crime Scenes, including "A Jury of Her Peers"		X			X	X	Instructions

Mock Trial

Subjects: English, Science, Social Studies

OVERVIEW

By conducting a mock trial, teachers may integrate Get A Clue with English and social studies. Students will investigate the American legal system, while practicing writing and public speaking skills. Teachers may use the scenario presented in Get A Clue, create their own scenario for trial, or reenact a famous historical or literary trial or investigation.

By reenacting a famous historical trial, a teacher may integrate US or World History into the lesson. Trial summaries, testimony, transcripts, and photos of evidence for many famous trials may be found at Attorney Douglas Linder's website "Famous Trials" (http://www.law.umkc.edu/faculty/projects/ftrials/ftrials.htm). The trial of Lizzie Borden would work well with Get A Clue. Attorney Linder provides photos and autopsy reports of the victims in the Lizzie Borden case.

English teachers may wish to adapt a literary trial or investigation. An example using the Russian novel The Brothers Karamazov, that was created and implemented by Robin Gore of West Bladen High School, is provided here.

RESOURCES

Hambouz, Annissa & Kahn, Javaid (2003, July 21). *Crime Time: Exploring the Fundamentals of American Criminal Justice*. Retrieved August 11, 2006, from The New York Times Web site: http://www.nytimes.com/learning/teachers/lessons/20030721monday.html?searchpv=learning_lessons

Linder, Douglas O. (2006). *Famous Trials*. Retrieved August 11, 2006, from University of Missouri-Kansas City School of Law Web site: http://www.law.umkc.edu/faculty/projects/ftrials/ftrials.htm

(2006). *Stages of a Criminal Case*. Retrieved August 11, 2006, from FindLaw for the Public Web site: http://criminal.findlaw.com/crimes/criminal_stages.html

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(2006). *The Innocence Project*. Retrieved August 11, 2006, Web site: http://www.innocenceproject.org/

(2006, July 26). *Street Law at the UW School of Law*. Retrieved August 11, 2006, from UW School of Law Web site: http://www.law.washington.edu/streetlaw/lessons.html

ACTIVITIES/PROCEDURES

I. Making Observations and Collecting Physical Evidence: This will vary depending on which scenario you choose for the class. If you construct a scenario and have the time, evidence may be "planted" in a mock crime scene. If you are following a famous case in history or a crime in literature, the students may look for evidence in descriptions of the crime scene. (Teachers may wish to invite local speakers, such as police detectives of members of the SBI, to speak about the methodology of investigating a crime scene.)

- a. Common crime scene evidence
  - i. Blood Evidence – if an historical or literary trial is used, the teacher may need to decide ahead of time what the DNA evidence will actually say. Even if the evidence is conclusive, there are usually mitigating factors that would give both the prosecution and defense plenty to work with.
  - ii. Fingerprints
  - iii. Fibers – hair, clothing
  - iv. Weapon
  - v. Shoeprints
  - vi. Victim

II. Preparing for Trial

- a. Divide students into two teams. One team will work for the prosecution, and one will work for the defense.

i. Students work together to compose an opening statement for their side. (Worksheets for preparing opening statements are available at http://www.law.washington.edu/streetlaw/lessons.html under "Mock Trial Preparation.")
    1. Guidelines on the length and scope of the opening statement will be determined by the teacher.
    2. The opening statement will be submitted to the teacher, and given back to the group with suggestions and edits.
    3. Students will choose which member of their team will present the statement.

ii. Students will also compile a list of witnesses that will be testifying for their "side."
    1. This list will be given to the teacher and then shared with the opposing side.
    2. Students will decide which member of their group will play the role of each of the witnesses.

iii. Student work together to compose a list of questions to ask each of the witnesses. The teacher may or may not wish to review this list before the "trial."

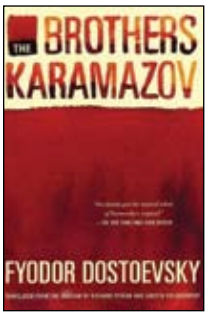
III. Classroom Trial
- a. Choosing a Jury
    - i. Instead of a jury verdict, students may be required to write a paper, after the trial, which argues for either guilt or innocence and discusses the American legal system.
- b. Opening Statements
- c. Witness Testimony and Cross-Examination
- d. Closing Arguments
    - i. A closing argument could be assigned as individual homework or as a group project. Teachers may wish to follow the same guidelines used for the opening statement.
- e. Jury Deliberation and Verdict
    - i. Instead of a jury verdict, students may be required to write a paper, after the trial, which argues for either guilt or innocence and discusses the American legal system.

Integrating Forensics, Civics, and World Literature: The Brothers Karamazov

Created by Robin Gore, West Bladen High School

This integrated activity is based on The Brothers Karamazov, by the Russian novelist Fyodor Dostoevsky. It is designed to integrate English II (World Literature), Biology, and Civics classes.

This activity addresses a number of Competency Goals from the North Carolina Standard Course of Study for English II, including Competency Goal Two, which focuses on evaluation of problems and cause-and-effect relationships; Competency Goal Three, which encourages students to gather information to prove a particular point; and Competency Goal Five, which contributes to students' understanding of world literature by asking them to engage in interpretation and analysis. In the Biology curriculum, it can be applied to Competency Goal Two, in which students assess the application of DNA technology to forensics, as well as Competency Goal Five's focus on the relationship between heredity and the environment.



PRE-LAB ACTIVITIES

1. Have students read Chapter One and do a pedigree of the family, making sure to include any genetic disorders that may occur. Add to this as you read further.

2. Do a character analysis of each character in the book.

3. As students read, have them keep a journal of the main story line and secondary characters.

4. Have students keep a chart of any evidence that was used to prosecute Dmitri. Determine what forms of evidence might be used if the crime occurred today.

LAB

The DESTINY module Get A Clue can be used here. We set it up as if we were analyzing Fyodor's blood from the Karamazov crime scene. In this adaptation, Get A Clue's "Suspect One" becomes DNA retrieved from the hair on the paperweight, and "Suspect Two" is blood from the pestle.

POST-LAB

Set up a mock trial to retry Dmitri, based on new DNA evidence. This would be an opportunity to integrate with a Civic's class.

Where can we find forensic evidence in the book? Where can we find DNA evidence?

1. Brass pestle (at the scene of Grigory's assault & Fyodor's murder)
2. Paperweight (It has been found! Ivan & Grushenka went searching for it after Ivan recovered from his breakdown. Smerdyakov had it hidden in the cellar near the sight of his original seizure. The paperweight is new evidence based on Ivan's recollection of the late Smerdyakov's confession.)
3. Dmitri's Handkerchief (dropped at Pyotr Perhotin's house)
4. Dmitri's shirt and coat
5. Plotnikov's washbasin (Double-check this to make sure that the washbasin wasn't at Pyotr Perhotin's house.)
6. Fyodor's nightgown

Whose DNA do we need to collect?

1. DNA on the pestle (from the hair fibers)
2. DNA on the paperweight (from blood)
3. Fyodor's DNA (We will have to exhume his body to do this.)
4. Grigory's DNA (The *Destiny* Bus helped us complete this task. It was learned through the DNA that the pestle did not have Fyodor's blood on it and the paperweight did, proving Smerdyakov's confession to be true.)

Whose fingerprints do we need to collect?

1. Dmitri's fingerprints
2. Smerdyakov's fingerprints (We will also have to exhume Smerdyakov's body to be able to lift his fingerprints. If his body is too badly decomposed, we will need to look at his thumb prints on his birth certificate if that can be found anywhere.)

What has happened since the first trial?

The bodies of Fyodor and Smerdyakov have been exhumed for the forensic evidence. Fyodor's skull is studied to check the blunt trauma wounds. After Ivan gets over his brain fever, he goes to Fetyukovitch and tells him the specifics of Smerdyakov's confession about the paperweight. He, Grushenka, and several men go to the Karamazov home and find the paperweight weapon. (Note: Ivan had already handed over the 3,000 rubles to the president of the court as evidence.)

Cross-examination
- Trifon Borissovitch: question about taxes and money management
- Dr. Herzenstube: possibly misunderstood the patient because of the doctor's foreign (German) background, and the question of what he originally said
- Moscow doctor: He thinks Dmitri is abnormal. He thinks Dmitri doesn't have the power to control his morbid impulse that possessed him and possibly made him insane.
- Dr. Varvinsky: He thought Dmitri was normal. He has compassion for Dmitri because he once gave him a pound of nuts when Dmitri was a ragged, pitiful little boy.

Money
- 5,000 roubles in 1870 = $33,234 today (embezzled money)
- 3,000 roubles = $19,940 (Katerina's money that Dmitri squandered twice on Grushenka)
- 200 roubles = $1,329 (money offered to Snegiryov)
- 25 roubles = $166 (doctor visit)
- 10 roubles = $66 (pawned pistols)
- 40,000 roubles = $265,870 (each legitimate child's inheritance)
- 120,000 roubles = $797,612 (total estate to inherit)
- 10,000 roubles = $66,456 (Ivan's money for Dmitri to escape)

Malta, Marfa, Foma
- Malta Ignatyevna woke up when she heard Smerdyakov screaming in one of his fits.
- Malfa called out to Grigory but realized that he was gone.
- She went out into the garden following Grigory's groans. She found him 20 paces from where he'd been knocked down with the brass pestle by Dmitri.
- She ran to Fyodor's window when she saw it was open. She screamed for help.
- She noticed Fyodor lying motionless, bloody, face down. He looked dead.
- She rushed away from the window, ran out of the garden, drew the bolt off of the big gate, and ran to Malfa's (Smerdyakov's girlfriend's) house. (Later, Marfa revealed that around 8:00 she had heard someone scream the word "Parricide!")
- Malta, Marfa & Foma went to the garden and carried Grigory to the lodge, bandaged his head, and then Grigory sent the ladies into the master's house.

Grushenka
- She said that she was sure that Dmitri had spent 3,000 roubles on during the first time in Mokroe.

Pyotr Ilyitch Perhotin
- Dmitri came to his house at 9:00 with blood on his clothes, hands, and handkerchief.
- Dmitri demanded his pistols.
- He saw Dmitri write a note.
- Dmitri and Pyotr went to Plotnikov's shop and Dmitri spent a lot of money on food and wine.
- Pyotr got halfway home and he decided to go to Grushenka's house (which was actually the widow Morozov's house). There he asked Fenya what she saw. Fenya told him she saw blood dripping from Dmitri the second time he came to her house. She told Pyotr that she knew that Dmitri had gone to Mokroe to kill Grushenka.
- Instead of going straight to the police captain's home, he goes to ask Madame Hohlakov about the money that Dmitri said she'd given to him.
- Madame Hohlakov told him the bit about the gold-mines and that she did not give Dmitri any money. She even wrote a little note to that effect on p. 455. He took that note with him to Mihail Makarovitch's house, but when he got there the three women had beaten him there with the fact that Fyodor was dead.

Precedence for the use of DNA evidence to reopen a criminal case

Through the diligent work of Fetyukovitch (Dmitri's Moscow defense attorney) with the arguments that new DNA evidence could prove Dmitri's innocence, the judge has agreed during the appeal for a re-trial. The re-trial date will depend on the court's backlogged calendar. Many old cases have been reopened recently because of DNA evidence.

Each of you will research to find out about and briefly summarize cases that would help Fetyukovitch in his appeal argument. Use the Internet, magazines, the news, or any other source to help him build his case. Behind each example, please state where you got the example from (e.g., Internet).

Examples of the kinds of cases you can investigate:

- When has DNA evidence has been used to find the right perpetrator? Night stalker case.
- When has DNA evidence regrettably been used too late, revealing that a person was wrongly executed for a crime the individual didn't commit?
- When has DNA evidence been successfully used in a court of law to prove the innocence of a person wrongly convicted of a crime?

Literary Crime Scenes

Following the model provided in "Integrating Forensics, Civics, and World Literature: The Brothers Karamazov," teachers may choose other works of literature that involve crime scenes. A list of possibilities, with a description of the pertinent crime scenes, is provided below.

AMERICAN LITERATURE

William Faulkner – *A Rose for Emily*

This tale ends with the discovery of a skeleton in a locked room of a home whose elderly owner, Emily Grierson, has now died. On the indented pillow next to the remains is a single, long, iron-gray hair. All of the evidence surrounding the skeleton is circumstantial.

F. Scott Fitzgerald – *The Great Gatsby*

A woman is killed in a hit and run accident. The car involved actually belonged to Gatsby but was being driven by someone else. Gatsby is later murdered by the husband of the hit-and-run victim, in a case of mistaken identity.

Susan Glaspell – *A Jury of Her Peers*

Local farmer Mr. Wright is found strangled to death in his home. The wife is arrested, and the sheriff and his friend spend much of the story looking for evidence. In the meantime, the sheriff's and friend's wives are to gather clothing for the arrested woman. The women see plenty of evidence that Mrs. Wright has been abused throughout her marriage. They take it upon themselves to conceal evidence that would implicate her in his strangling.

Harper Lee – *To Kill a Mockingbird*

Tom Robinson, a black man, is placed on trial for raping Mayella Ewell, a white woman. Although significant evidence is presented to prove that Tom is innocent, he is convicted by the all-white jury.

BRITISH LITERATURE

Charles Dickens – *Our Mutual Friend*

A body is found in the Thames that is believed to be that of the young John Harmon, heir to his father's profitable heaps of refuse. Later in the novel it is revealed that a mysterious young man, going by the name of John Rokesmith, is actually the young Harmon, who has survived attempted murder and is living incognito.

George Eliot – *Adam Bede*

An unmarried woman gives birth to a baby and leaves it do die. She is convicted of the baby's murder but her sentence is commuted when the father discovers her plight.

Robert Louis Stevenson – *The Strange Case of Dr. Jekyll and Mr. Hyde*

A client of the lawyer Utterson is brutally murdered. The murder weapon is a cane that Utterson had given to Dr. Jekyll. Utterson's curiosity and subsequent investigations help to reveal "the strange case of Dr. Jekyll and Mr. Hyde."

William Shakespeare –

Macbeth

Macbeth murders King Duncan, and Lady Macbeth frames his servants for the deed.

Hamlet

The king of Denmark dies suddenly, of a purported snake bite. His son, Prince Hamlet, suspects that the king's brother, now King Claudius, murdered his late father.

WORLD LITERATURE

Fiodor Dostoevskii –

Crime and Punishment

An old pawnbroker and her pregnant sister are brutally murdered. While the reader knows the identity of the murderer, the police inspector spends much of the novel trying to make his case.

The Possessed

A group of revolutionaries conspire and commit murder.

The Brothers Karamazov

This novel contains patricide, a murder trial and a question of paternity.

Sophocles – *Oedipus Rex*

In trying to escape prophecy, Oedipus fulfills it. He has murdered his father and born children by his mother.

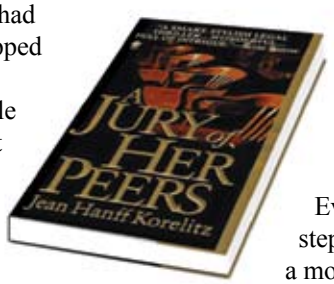
A JURY OF HER PEERS

By Susan Glaspell

Originally published in The Best Short Stories of 1917. Ed. Edward J. O'Brien. Boston: Small, Maynard & Company, 1918. 256-282.

When Martha Hale opened the storm-door and got a cut of the north wind, she ran back for her big woolen scarf. As she hurriedly wound that round her head her eye made a scandalized sweep of her kitchen. It was no ordinary thing that called her away – it was probably further from ordinary than anything that had ever happened in Dickson County. But what her eye took in was that her kitchen was in no shape for leaving: her bread all ready for mixing, half the flour sifted and half unsifted.

She hated to see things half done; but she had been at that when the team from town stopped to get Mr. Hale, and then the sheriff came running in to say his wife wished Mrs. Hale would come too – adding, with a grin, that he guessed she was getting scary and wanted another woman along. So she had dropped everything right where it was.



"Martha!" now came her husband's impatient voice. "Don't keep folks waiting out here in the cold."

She again opened the storm-door, and this time joined the three men and the one woman waiting for her in the big two-seated buggy.

After she had the robes tucked around her she took another look at the woman who sat beside her on the back seat. She had met Mrs. Peters the year before at the county fair, and the thing she remembered about her was that she didn't seem like a sheriff's wife. She was small and thin and didn't have a strong voice. Mrs. Gorman, sheriff's wife before Gorman went out and Peters came in, had a voice that somehow seemed to be backing up the law with every word. But if Mrs. Peters didn't look like a sheriff's wife, Peters made it up in looking like a sheriff. He was to a dot the kind of man who could get himself elected sheriff – a heavy man with a big voice, who was particularly genial with the law-abiding, as if to make it plain that he knew the difference between criminals and non-criminals. And right there it came into Mrs. Hale's mind, with a stab, that this man who was so pleasant and lively with all of them was going to the Wrights' now as a sheriff.

"The country's not very pleasant this time of year," Mrs. Peters at last ventured, as if she felt they ought to be talking as well as the men.

Mrs. Hale scarcely finished her reply, for they had gone up a little hill and could see the Wright place now, and seeing it did not make her feel like talking. It looked very lonesome this cold March morning. It had always been a lonesome-looking place. It was down in a hollow, and the poplar trees around it were lonesome-looking trees. The men were looking at it and talking about what had happened. The county attorney was bending to one side of the buggy, and kept looking steadily at the place as they drew up to it.

"I'm glad you came with me," Mrs. Peters said nervously, as the two women were about to follow the men in through the kitchen door.

Even after she had her foot on the doorstep, her hand on the knob, Martha Hale had a moment of feeling she could not cross that threshold. And the reason it seemed she couldn't cross it now was simply because she hadn't crossed it before. Time and time again it had been in her mind, "I ought to go over and see Minnie Foster" – she still thought of her as Minnie Foster, though for twenty years she had been Mrs. Wright. And then there was always something to do and Minnie Foster would go from her mind. But now she could come.

The men went over to the stove. The women stood close together by the door. Young Henderson, the county attorney, turned around and said, "Come up to the fire, ladies."

Mrs. Peters took a step forward, then stopped. "I'm not cold," she said.

And so the two women stood by the door, at first not even so much as looking around the kitchen.

The men talked for a minute about what a good thing it was the sheriff had sent his deputy out that morning to make a fire for them, and then Sheriff Peters stepped back from the stove, unbuttoned his outer coat, and leaned his hands on the kitchen table in a way that seemed to mark the beginning of official business. "Now, Mr. Hale," he said in a sort of semi-official voice, "before we move things about, you tell Mr. Henderson just what it was you saw when you came here yesterday morning."

The county attorney was looking around the kitchen.

"By the way," he said, "has anything been moved?" He turned to the sheriff. "Are things just as you left them yesterday?"

Peters looked from cupboard to sink; from that to a small worn rocker a little to one side of the kitchen table.

"It's just the same."

"Somebody should have been left here yesterday," said the county attorney.

"Oh – yesterday," returned the sheriff, with a little gesture as of yesterday having been more than he could bear to think of. "When I had to send Frank to Morris Center for that man who went crazy – let me tell you. I had my hands full yesterday. I knew you could get back from Omaha by today, George, and as long as I went over everything here myself – "

"Well, Mr. Hale," said the county attorney, in a way of letting what was past and gone go, "tell just what happened when you came here yesterday morning."

Mrs. Hale, still leaning against the door, had that sinking feeling of the mother whose child is about to speak a piece. Lewis often wandered along and got things mixed up in a story. She hoped he would tell this straight and plain, and not say unnecessary things that would just make things harder for Minnie Foster. He didn't begin at once, and she noticed that he looked queer – as if standing in that kitchen and having to tell what he had seen there yesterday morning made him almost sick.

"Yes, Mr. Hale?" the county attorney reminded.

"Harry and I had started to town with a load of potatoes," Mrs. Hale's husband began.

Harry was Mrs. Hale's oldest boy. He wasn't with them now, for the very good reason that those potatoes never got to town yesterday and he was taking them this morning, so he hadn't been home when the sheriff stopped to say he wanted Mr. Hale to come over to the Wright place and tell the county attorney his story there, where he could point it all out. With all Mrs. Hale's other emotions came the fear now that maybe Harry wasn't dressed warm enough – they hadn't any of them realized how that north wind did bite.

"We come along this road," Hale was going on, with a motion of his hand to the road over which they had just come, "and as we got in sight of the house I says to Harry, 'I'm goin' to see if I can't get John Wright to take a telephone.' You see," he explained to Henderson, "unless I can get somebody to go in with me they won't come out this branch road except for a price I can't pay. I'd spoke to Wright about it once before; but he put me off, saying folks talked too much anyway, and all he asked was peace and quiet – guess you know about how much he talked himself. But I thought maybe if I went to the house and talked about it before his wife, and said all the women-folks liked the telephones, and that in this lonesome stretch of road it would be a good thing – well, I said to Harry that that was what I was going to say – though I said at the same time that I didn't know as what his wife wanted made much difference to John – "

Now there he was! – saying things he didn't need to say. Mrs. Hale tried to catch her husband's eye, but fortunately the county attorney interrupted with:

"Let's talk about that a little later, Mr. Hale. I do want to talk about that but, I'm anxious now to get along to just what happened when you got here."

When he began this time, it was very deliberately and carefully:

"I didn't see or hear anything. I knocked at the door. And still it was all quiet inside. I knew they must be up – it was past eight o'clock. So I knocked again, louder, and I thought I heard somebody say, 'Come in.' I wasn't sure – I'm not sure yet. But I opened the door – this door," jerking a hand toward the door by which the two women stood. "and there, in that rocker" – pointing to it – "sat Mrs. Wright."

Everyone in the kitchen looked at the rocker. It came into Mrs. Hale's mind that that rocker didn't look in the least like Minnie Foster – the Minnie Foster of twenty years before. It was a dingy red, with wooden rungs up the back, and the middle rung was gone, and the chair sagged to one side.

"How did she – look?" the county attorney was inquiring.

"Well," said Hale, "she looked – queer."

"How do you mean – queer?"

As he asked it he took out a note-book and pencil. Mrs.

Hale did not like the sight of that pencil. She kept her eye fixed on her husband, as if to keep him from saying unnecessary things that would go into that note-book and make trouble.

Hale did speak guardedly, as if the pencil had affected him too.

"Well, as if she didn't know what she was going to do next. And kind of – done up."

"How did she seem to feel about your coming?"

"Why, I don't think she minded – one way or other. She didn't pay much attention. I said, 'Ho' do, Mrs. Wright? It's cold, ain't it?' And she said. 'Is it?' – and went on pleatin' at her apron.

"Well, I was surprised. She didn't ask me to come up to the stove, or to sit down, but just set there, not even lookin' at me. And so I said: 'I want to see John.'

"And then she – laughed. I guess you would call it a laugh.

"I thought of Harry and the team outside, so I said, a little sharp, 'Can I see John?' 'No,' says she – kind of dull like. 'Ain't he home?' says I. Then she looked at me. 'Yes,' says she, 'he's home.' 'Then why can't I see him?' I asked her, out of patience with her now. 'Cause he's dead' says she, just as quiet and dull – and fell to pleatin' her apron. 'Dead?' says, I, like you do when you can't take in what you've heard.

"She just nodded her head, not getting a bit excited, but rockin' back and forth.

"'Why – where is he?' says I, not knowing what to say.

"She just pointed upstairs – like this" – pointing to the room above.

"I got up, with the idea of going up there myself. By this time I – didn't know what to do. I walked from there to here; then I says: 'Why, what did he die of?'

"'He died of a rope around his neck,' says she; and just went on pleatin' at her apron."

Hale stopped speaking, and stood staring at the rocker, as if he were still seeing the woman who had sat there the morning before. Nobody spoke; it was as if every one were seeing the woman who had sat there the morning before.

"And what did you do then?" the county attorney at last broke the silence.

"I went out and called Harry. I thought I might – need help. I got Harry in, and we went upstairs." His voice fell almost to a whisper. "There he was – lying over the – "

"I think I'd rather have you go into that upstairs," the county attorney interrupted, "where you can point it all out. Just go on now with the rest of the story."

"Well, my first thought was to get that rope off. It looked – "

He stopped, his face twitching.

"But Harry, he went up to him, and he said. 'No, he's dead all right, and we'd better not touch anything.' So we went downstairs.

"She was still sitting that same way. 'Has anybody been notified?' I asked. 'No, says she, unconcerned.

"'Who did this, Mrs. Wright?' said Harry. He said it businesslike, and she stopped pleatin' at her apron. 'I don't know,' she says. 'You don't know?' says Harry. 'Weren't you sleepin' in the bed with him?' 'Yes,' says she, 'but I was on the inside. 'Somebody slipped a rope round his neck and strangled him, and you didn't wake up?' says Harry. 'I didn't wake up,' she said after him.

"We may have looked as if we didn't see how that could be, for after a minute she said, 'I sleep sound.'

"Harry was going to ask her more questions, but I said maybe that weren't our business; maybe we ought to let her tell her story first to the coroner or the sheriff. So Harry went fast as he could over to High Road – the Rivers' place, where there's a telephone."

"And what did she do when she knew you had gone for the coroner?" The attorney got his pencil in his hand all ready for writing.

"She moved from that chair to this one over here" – Hale pointed to a small chair in the corner – "and just sat there with her hands held together and lookin down. I got a feeling that I ought to make some conversation, so I said I had come in to see if John wanted to put in a telephone; and at that she started to laugh, and then she stopped and looked at me – scared."

At the sound of a moving pencil the man who was telling the story looked up.

"I dunno – maybe it wasn't scared," he hastened: "I wouldn't like to say it was. Soon Harry got back, and then Dr. Lloyd came, and you, Mr. Peters, and so I guess that's all I know that you don't."

He said that last with relief, and moved a little, as if relaxing. Everyone moved a little. The county attorney walked toward the stair door.

"I guess we'll go upstairs first – then out to the barn and around there."

He paused and looked around the kitchen.

"You're convinced there was nothing important here?" he asked the sheriff. "Nothing that would – point to any motive?"

The sheriff too looked all around, as if to re-convince himself.

"Nothing here but kitchen things," he said, with a little laugh for the insignificance of kitchen things.

The county attorney was looking at the cupboard – a peculiar, ungainly structure, half closet and half cupboard, the upper part of it being built in the wall, and the lower part just the old-fashioned kitchen cupboard. As if its queerness attracted him, he got a chair and opened the upper part and looked in. After a moment he drew his hand away sticky.

"Here's a nice mess," he said resentfully.

The two women had drawn nearer, and now the sheriff's wife spoke.

"Oh – her fruit," she said, looking to Mrs. Hale for sympathetic understanding.

She turned back to the county attorney and explained: "She worried about that when it turned so cold last night. She said the fire would go out and her jars might burst."

Mrs. Peters' husband broke into a laugh.

"Well, can you beat the women! Held for murder, and worrying about her preserves!"

The young attorney set his lips.

"I guess before we're through with her she may have something more serious than preserves to worry about."

"Oh, well," said Mrs. Hale's husband, with good-natured superiority, "women are used to worrying over trifles."

The two women moved a little closer together. Neither of them spoke. The county attorney seemed suddenly to remember his manners – and think of his future.

"And yet," said he, with the gallantry of a young politician. "for all their worries, what would we do without the ladies?"

The women did not speak, did not unbend. He went to the sink and began washing his hands. He turned to wipe them on the roller towel – whirled it for a cleaner place.

"Dirty towelsl Not much of a housekeeper, would you say, ladies?"

He kicked his foot against some dirty pans under the sink.

"There's a great deal of work to be done on a farm," said Mrs. Hale stiffly.

"To be sure. And yet" – with a little bow to her – 'I know there are some Dickson County farm-houses that do not have such roller towels." He gave it a pull to expose its full length again.

"Those towels get dirty awful quick. Men's hands aren't always as clean as they might be.

"Ah, loyal to your sex, I see," he laughed. He stopped and gave her a keen look, "But you and Mrs. Wright were neighbors. I suppose you were friends, too."

Martha Hale shook her head.

"I've seen little enough of her of late years. I've not been in this house – it's more than a year."

"And why was that? You didn't like her?"

"I liked her well enough," she replied with spirit. "Farmers' wives have their hands full, Mr. Henderson. And then – " She looked around the kitchen.

"Yes?" he encouraged.

"It never seemed a very cheerful place," said she, more to herself than to him.

"No," he agreed; "I don't think anyone would call it cheerful. I shouldn't say she had the home-making instinct."

"Well, I don't know as Wright had, either," she muttered.

"You mean they didn't get on very well?" he was quick to ask.

"No; I don't mean anything," she answered, with decision. As she turned a lit- tle away from him, she added: "But I don't think a place would be any the cheerfuller for John Wright's bein' in it."

"I'd like to talk to you about that a little later, Mrs. Hale," he said. "I'm anxious to get the lay of things upstairs now."

He moved toward the stair door, followed by the two men.

"I suppose anything Mrs. Peters does'll be all right?" the sheriff inquired. "She was to take in some clothes for her, you know – and a few little things. We left in such a hurry yesterday."

The county attorney looked at the two women they were leaving alone there among the kitchen things.

"Yes – Mrs. Peters," he said, his glance resting on the woman who was not Mrs. Peters, the big farmer woman who stood behind the sheriff's wife. "Of course Mrs. Peters is one of us," he said, in a manner of entrusting responsibility. "And keep your eye out, Mrs. Peters, for anything that might be of use. No telling; you women might come upon a clue to the motive – and that's the thing we need."

Mr. Hale rubbed his face after the fashion of a showman getting ready for a pleasantry.

"But would the women know a clue if they did come upon it?" he said; and, having delivered himself of this, he followed the others through the stair door.

The women stood motionless and silent, listening to the footsteps, first upon the stairs, then in the room above them.

Then, as if releasing herself from something strange. Mrs. Hale began to arrange the dirty pans under the sink, which the county attorney's disdainful push of the foot had deranged.

"I'd hate to have men comin' into my kitchen," she said testily – "snoopin' round and criticizin'."

"Of course it's no more than their duty," said the sheriff's wife, in her manner of timid acquiescence.

"Duty's all right," replied Mrs. Hale bluffly; "but I guess that deputy sheriff that come out to make the fire might have got a little of this on." She gave the roller towel a pull. 'Wish I'd thought of that sooner! Seems mean to talk about her for not having things slicked up, when she had to come away in such a hurry."

She looked around the kitchen. Certainly it was not "slicked up." Her eye was held by a bucket of sugar on a low shelf. The cover was off the wooden bucket, and beside it was a paper bag – half full.

Mrs. HaIe moved toward it.

"She was putting this in there," she said to herself – slowly.

She thought of the flour in her kitchen at home – half sifted, half not sifted. She had been interrupted, and had left things half done. What had interrupted Minnie Foster? Why had that work been left half done? She made a move as if to finish it, – unfinished things always bothered her, – and then she glanced around and saw that Mrs. Peters was watching her – and she didn't want Mrs. Peters to get that feeling she had got of work begun and then – for some reason – not finished.

"It's a shame about her fruit," she said, and walked toward the cupboard that the county attorney had opened, and got on the chair, murmuring: "I wonder if it's all gone."

It was a sorry enough looking sight, but "Here's one that's all right," she said at last. She held it toward the light. "This is cherries, too." She looked again. "I declare I believe that's the only one."

With a sigh, she got down from the chair, went to the sink, and wiped off the bottle.

"She'Il feel awful bad, after all her hard work in the hot weather. I remember the afternoon I put up my cherries last summer.

She set the bottle on the table, and, with another sigh, started to sit down in the rocker. But she did not sit down. Something kept her from sitting down in that chair. She straightened – stepped back, and, half turned

away, stood looking at it, seeing the woman who had sat there "pleatin' at her apron."

The thin voice of the sheriff's wife broke in upon her: "I must be getting those things from the front-room closet." She opened the door into the other room, started in, stepped back. "You coming with me, Mrs. Hale?" she asked nervously. "You – you could help me get them."

They were soon back – the stark coldness of that shut-up room was not a thing to linger in.

"My!" said Mrs. Peters, dropping the things on the table and hurrying to the stove.

Mrs. Hale stood examining the clothes the woman who was being detained in town had said she wanted.

"Wright was close!" she exclaimed, holding up a shabby black skirt that bore the marks of much making over. "I think maybe that's why she kept so much to herself. I s'pose she felt she couldn't do her part; and then, you don't enjoy things when you feel shabby. She used to wear pretty clothes and be lively – when she was Minnie Foster, one of the town girls, singing in the choir. But that – oh, that was twenty years ago."

With a carefulness in which there was something tender, she folded the shabby clothes and piled them at one corner of the table. She looked up at Mrs. Peters, and there was something in the other woman's look that irritated her.

"She don't care," she said to herself. "Much difference it makes to her whether Minnie Foster had pretty clothes when she was a girl."

Then she looked again, and she wasn't so sure; in fact, she hadn't at any time been perfectly sure about Mrs. Peters. She had that shrinking manner, and yet her eyes looked as if they could see a long way into things.

"This all you was to take in?" asked Mrs. Hale.

"No," said the sheriffs wife; "she said she wanted an apron. Funny thing to want, " she ventured in her nervous little way, "for there's not much to get you dirty in jail, goodness knows. But I suppose just to make her feel more natural. If you're used to wearing an apron – . She said they were in the bottom drawer of this cupboard. Yes – here they are. And then her little shawl that always hung on the stair door."

She took the small gray shawl from behind the door leading upstairs, and stood a minute looking at it.

Suddenly Mrs. Hale took a quick step toward the other woman, "Mrs. Peters!"

"Yes, Mrs. Hale?"

"Do you think she – did it?'

A frightened look blurred the other thing in Mrs. Peters' eyes.

"Oh, I don't know," she said, in a voice that seemed to shink away from the subject.

"Well, I don't think she did," affirmed Mrs. Hale stoutly. "Asking for an apron, and her little shawl. Worryin' about her fruit."

"Mr. Peters says – ." Footsteps were heard in the room above; she stopped, looked up, then went on in a lowered voice: "Mr. Peters says – it looks bad for her. Mr. Henderson is awful sarcastic in a speech, and he's going to make fun of her saying she didn't – wake up."

For a moment Mrs. Hale had no answer. Then, "Well, I guess John Wright didn't wake up – when they was slippin' that rope under his neck," she muttered.

"No, it's strange," breathed Mrs. Peters. "They think it was such a – funny way to kill a man."

She began to laugh; at sound of the laugh, abruptly stopped.

"That's just what Mr. Hale said," said Mrs. Hale, in a resolutely natural voice. "There was a gun in the house. He says that's what he can't understand."

"Mr. Henderson said, coming out, that what was needed for the case was a motive. Something to show anger – or sudden feeling."

'Well, I don't see any signs of anger around here," said Mrs. Hale, "I don't – " She stopped. It was as if her mind tripped on something. Her eye was caught by a dish-towel in the middle of the kitchen table. Slowly she moved toward the table. One half of it was wiped clean, the other half messy. Her eyes made a slow, almost unwilling turn to the bucket of sugar and the half empty bag beside it. Things begun – and not finished.

After a moment she stepped back, and said, in that man-

ner of releasing herself:

"Wonder how they're finding things upstairs? I hope she had it a little more red up up there. You know," – she paused, and feeling gathered, – "it seems kind of sneaking: locking her up in town and coming out here to get her own house to turn against her!"

"But, Mrs. Hale," said the sheriff's wife, "the law is the law."

"I s'pose 'tis," answered Mrs. Hale shortly.

She turned to the stove, saying something about that fire not being much to brag of. She worked with it a minute, and when she straightened up she said aggressively:

"The law is the law – and a bad stove is a bad stove. How'd you like to cook on this?" – pointing with the poker to the broken lining. She opened the oven door and started to express her opinion of the oven; but she was swept into her own thoughts, thinking of what it would mean, year after year, to have that stove to wrestle with. The thought of Minnie Foster trying to bake in that oven – and the thought of her never going over to see Minnie Foster – .

She was startled by hearing Mrs. Peters say: "A person gets discouraged – and loses heart."

The sheriff's wife had looked from the stove to the sink – to the pail of water which had been carried in from outside. The two women stood there silent, above them the footsteps of the men who were looking for evidence against the woman who had worked in that kitchen. That look of seeing into things, of seeing through a thing to something else, was in the eyes of the sheriff's wife now. When Mrs. Hale next spoke to her, it was gently:

"Better loosen up your things, Mrs. Peters. We'll not feel them when we go out."

Mrs. Peters went to the back of the room to hang up the fur tippet she was wearing. A moment later she exclaimed, "Why, she was piecing a quilt," and held up a large sewing basket piled high with quilt pieces.

Mrs. Hale spread some of the blocks on the table.

"It's log-cabin pattern," she said, putting several of them together, "Pretty, isn't it?"

They were so engaged with the quilt that they did not hear the footsteps on the stairs. Just as the stair door opened Mrs. Hale was saying:

"Do you suppose she was going to quilt it or just knot it?"

The sheriff threw up his hands.

"They wonder whether she was going to quilt it or just knot it!"

There was a laugh for the ways of women, a warming of hands over the stove, and then the county attorney said briskly:

"Well, let's go right out to the barn and get that cleared up."

"I don't see as there's anything so strange," Mrs. Hale said resentfully, after the outside door had closed on the three men – "our taking up our time with little things while we're waiting for them to get the evidence. I don't see as it's anything to laugh about."

"Of course they've got awful important things on their minds," said the sheriff's wife apologetically.

They returned to an inspection of the block for the quilt. Mrs. Hale was looking at the fine, even sewing, and preoccupied with thoughts of the woman who had done that sewing, when she heard the sheriff's wife say, in a queer tone:

"Why, look at this one."

She turned to take the block held out to her.

"The sewing," said Mrs. Peters, in a troubled way, "All the rest of them have been so nice and even – but – this one. Why, it looks as if she didn't know what she was about!"

Their eyes met – something flashed to life, passed between them; then, as if with an effort, they seemed to pull away from each other. A moment Mrs. Hale sat there, her hands folded over that sewing which was so unlike all the rest of the sewing. Then she had pulled a knot and drawn the threads.

"Oh, what are you doing, Mrs. Hale?" asked the sheriff's wife, startled.

"Just pulling out a stitch or two that's not sewed very good," said Mrs. Hale mildly.

"I don't think we ought to touch things," Mrs. Peters said, a little helplessly.

"I'll just finish up this end," answered Mrs. Hale, still in that mild, matter-of-fact fashion.

She threaded a needle and started to replace bad sewing with good. For a little while she sewed in silence. Then, in that thin, timid voice, she heard:

"Mrs. Hale!"

"Yes, Mrs. Peters?"

'What do you suppose she was so – nervous about?"

"Oh, I don't know," said Mrs. Hale, as if dismissing a thing not important enough to spend much time on. "I don't know as she was – nervous. I sew awful queer sometimes when I'm just tired."

She cut a thread, and out of the corner of her eye looked up at Mrs. Peters. The small, lean face of the sheriff's wife seemed to have tightened up. Her eyes had that look of peering into something. But next moment she moved, and said in her thin, indecisive way:

'Well, I must get those clothes wrapped. They may be through sooner than we think. I wonder where I could find a piece of paper – and string."

"In that cupboard, maybe," suggested to Mrs. Hale, after a glance around.

One piece of the crazy sewing remained unripped. Mrs. Peter's back turned, Martha Hale now scrutinized that piece, compared it with the dainty, accurate sewing of the other blocks. The difference was startling. Holding this block made her feel queer, as if the distracted thoughts of the woman who had perhaps turned to it to try and quiet herself were communicating themselves to her.

Mrs. Peters' voice roused her.

"Here's a bird-cage," she said. "Did she have a bird, Mrs. Hale?"

'Why, I don't know whether she did or not." She turned to look at the cage Mrs. Peters was holding up. "I've not been here in so long." She sighed. "There was a man round last year selling canaries cheap – but I don't know as she took one. Maybe she did. She used to sing real pretty herself."

Mrs. Peters looked around the kitchen.

"Seems kind of funny to think of a bird here." She half laughed – an attempt to put up a barrier. "But she must have had one – or why would she have a cage? I wonder what happened to it."

"I suppose maybe the cat got it," suggested Mrs. Hale, resuming her sewing.

"No; she didn't have a cat. She's got that feeling some people have about cats – being afraid of them. When they brought her to our house yesterday, my cat got in the room, and she was real upset and asked me to take it out."

"My sister Bessie was like that," laughed Mrs. Hale.

The sheriff's wife did not reply. The silence made Mrs. Hale turn round. Mrs. Peters was examining the bird-cage.

"Look at this door," she said slowly. "It's broke. One hinge has been pulled apart."

Mrs. Hale came nearer.

"Looks as if someone must have been – rough with it."

Again their eyes met – startled, questioning, apprehensive. For a moment neither spoke nor stirred. Then Mrs. Hale, turning away, said brusquely:

"If they're going to find any evidence, I wish they'd be about it. I don't like this place."

"But I'm awful glad you came with me, Mrs. Hale." Mrs. Peters put the bird-cage on the table and sat down. "It would be lonesome for me – sitting here alone."

"Yes, it would, wouldn't it?" agreed Mrs. Hale, a certain determined naturalness in her voice. She had picked up the sewing, but now it dropped in her lap, and she murmured in a different voice: "But I tell you what I do wish, Mrs. Peters. I wish I had come over sometimes when she was here. I wish – I had."

"But of course you were awful busy, Mrs. Hale. Your house – and your children."

"I could've come," retorted Mrs. Hale shortly. "I stayed away because it weren't cheerful – and that's why I ought to have come. I" – she looked around – "I've never liked this place. Maybe because it's down in a hollow and you don't see the road. I don't know what

it is, but it's a lonesome place, and always was. I wish I had come over to see Minnie Foster sometimes. I can see now – " She did not put it into words.

"Well, you mustn't reproach yourself," counseled Mrs. Peters. "Somehow, we just don't see how it is with other folks till – something comes up."

"Not having children makes less work," mused Mrs. Hale, after a silence, "but it makes a quiet house – and Wright out to work all day – and no company when he did come in. Did you know John Wright, Mrs. Peters?"

"Not to know him. I've seen him in town. They say he was a good man."

"Yes – good," conceded John Wright's neighbor grimly. "He didn't drink, and kept his word as well as most, I guess, and paid his debts. But he was a hard man, Mrs. Peters. Just to pass the time of day with him – ." She stopped, shivered a little. "Like a raw wind that gets to the bone." Her eye fell upon the cage on the table before her, and she added, almost bitterly: "I should think she would've wanted a bird!"

Suddenly she leaned forward, looking intently at the cage. "But what do you s'pose went wrong with it?"

"I don't know," returned Mrs. Peters; "unless it got sick and died."

But after she said it she reached over and swung the broken door. Both women watched it as if somehow held by it.

"You didn't know – her?" Mrs. Hale asked, a gentler note in her voice.

"Not till they brought her yesterday," said the sheriff's wife.

"She – come to think of it, she was kind of like a bird herself. Real sweet and pretty, but kind of timid and – fluttery. How – she – did – change."

That held her for a long time. Finally, as if struck with a happy thought and relieved to get back to everyday things, she exclaimed:

"Tell you what, Mrs. Peters, why don't you take the quilt in with you? It might take up her mind."

"Why, I think that's a real nice idea, Mrs. Hale," agreed the sheriff's wife, as if she too were glad to come into the atmosphere of a simple kindness. "There couldn't possibly be any objection to that, could there? Now, just what will I take? I wonder if her patches are in here – and her things?"

They turned to the sewing basket.

"Here's some red," said Mrs. Hale, bringing out a roll of cloth. Underneath that was a box. "Here, maybe her scissors are in here – and her things." She held it up. "What a pretty box! I'll warrant that was something she had a long time ago – when she was a girl."

She held it in her hand a moment; then, with a little sigh, opened it.

Instantly her hand went to her nose.

"Why – !"

Mrs. Peters drew nearer – then turned away.

"There's something wrapped up in this piece of silk," faltered Mrs. Hale.

"This isn't her scissors," said Mrs. Peters, in a shrinking voice.

Her hand not steady, Mrs. Hale raised the piece of silk. "Oh, Mrs. Peters!" she cried. "It's – "

Mrs. Peters bent closer.

"It's the bird," she whispered.

"But, Mrs. Peters!" cried Mrs. Hale. "Look at it! Its neck – look at its neck! It's all – other side to."

She held the box away from her.

The sheriff's wife again bent closer.

"Somebody wrung its neck," said she, in a voice that was slow and deep.

And then again the eyes of the two women met – this time clung together in a look of dawning comprehension, of growing horror. Mrs. Peters looked from the dead bird to the broken door of the cage. Again their eyes met. And just then there was a sound at the outside door. Mrs. Hale slipped the box under the quilt pieces in the basket, and sank into the chair before it. Mrs. Peters stood holding to the table. The county attorney and the sheriff came in from outside.

"Well, ladies," said the county attorney, as one turning from serious things to little pleasantries, "have you decided whether she was going to quilt it or knot it?"

"We think," began the sheriff's wife in a flurried voice, "that she was going to – knot it."

He was too preoccupied to notice the change that came in her voice on that last.

"Well, that's very interesting, I'm sure," he said tolerantly. He caught sight of the bird-cage.

"Has the bird flown?"

"We think the cat got it," said Mrs. Hale in a voice curiously even.

He was walking up and down, as if thinking something out.

"Is there a cat?" he asked absently.

Mrs. Hale shot a look up at the sheriff's wife.

"Well, not now," said Mrs. Peters. "They're superstitious, you know; they Ieave."

She sank into her chair.

The county attorney did not heed her. "No sign at all of anyone having come in from the outside," he said to Peters, in the manner of continuing an interrupted conversation. "Their own rope. Now let's go upstairs again and go over it, picee by piece. It would have to have been someone who knew just the – "

The stair door closed behind them and their voices were lost.

The two women sat motionless, not looking at each other, but as if peering into something and at the same time holding back. When they spoke now it was as if they were afraid of what they were saying, but as if they could not help saying it.

"She liked the bird," said Martha Hale, low and slowly. "She was going to bury it in that pretty box."

When I was a girl," said Mrs. Peters, under her breath, "my kitten – there was a boy took a hatchet, and before my eyes – before I could get there – " She covered her face an instant. "If they hadn't held me back I would have" – she caught herself, looked upstairs where footsteps were heard, and finished weakly – "hurt him."

Then they sat without speaking or moving.

"I wonder how it would seem," Mrs. Hale at last began, as if feeling her way over strange ground – "never to have had any children around?" Her eyes made a slow sweep of the kitchen, as if seeing what that kitchen had meant through all the years "No, Wright wouldn't like the bird," she said after that – "a thing that sang. She used to sing. He killed that too." Her voice tightened.

Mrs. Peters moved uneasily.

"Of course we don't know who killed the bird."

"I knew John Wright," was Mrs. Hale's answer.

"It was an awful thing was done in this house that night, Mrs. Hale," said the sheriff's wife. "Killing a man while he slept – slipping a thing round his neck that choked the life out of him."

Mrs. Hale's hand went out to the bird cage.

"We don't know who killed him," whispered Mrs. Peters wildly. "We don't know."

Mrs. Hale had not moved. "If there had been years and years of – nothing, then a bird to sing to you, it would be awful – still – after the bird was still."

It was as if something within her not herself had spoken, and it found in Mrs. Peters something she did not know as herself.

"I know what stillness is," she said, in a queer, monotonous voice. "When we homesteaded in Dakota, and my first baby died – after he was two years old – and me with no other then – "

Mrs. Hale stirred.

"How soon do you suppose they'll be through looking for the evidence?"

"I know what stillness is," repeated Mrs. Peters, in just that same way. Then she too pulled back. "The law has got to punish crime, Mrs. Hale," she said in her tight little way.

"I wish you'd seen Minnie Foster," was the answer, "when she wore a white dress with blue ribbons, and stood up there in the choir and sang."

The picture of that girl, the fact that she had lived neighbor to that girl for twenty years, and had let her die for lack of life, was suddenly more than she could bear.

"Oh, I wish I'd come over here once in a while!" she cried. "That was a crime! Who's going to punish that?"

"We mustn't take on," said Mrs. Peters, with a frightened look toward the stairs.

"I might 'a' known she needed help! I tell you, it's queer, Mrs. Peters. We live close together, and we live far apart. We all go through the same things – it's all just a different kind of the same thing! If it weren't – why do you and I understand? Why do we know – what we know this minute?"

She dashed her hand across her eyes. Then, seeing the jar of fruit on the table she reached for it and choked out:

"If I was you I wouldn't tell her her fruit was gone! Tell her it ain't. Tell her it's all right – all of it. Here – take this in to prove it to her! She – she may never know whether it was broke or not."

She turned away.

Mrs. Peters reached out for the bottle of fruit as if she were glad to take it – as if touching a familiar thing, having something to do, could keep her from something else. She got up, looked about for something to wrap the fruit in, took a petticoat from the pile of clothes she had brought from the front room, and nervously started winding that round the bottle.

"My!" she began, in a high, false voice, "it's a good thing the men couldn't hear us! Getting all stirred up over a little thing like a – dead canary." She hurried over that. "As if that could have anything to do with – with – My, wouldn't they laugh?"

Footsteps were heard on the stairs.

"Maybe they would," muttered Mrs. Hale – "maybe they wouldn't."

"No, Peters," said the county attorney incisively; "it's all perfectly clear, except the reason for doing it. But you know juries when it comes to women. If there was some definite thing – something to show. Something to make a story about. A thing that would connect up with this clumsy way of doing it."

In a covert way Mrs. Hale looked at Mrs. Peters. Mrs. Peters was looking at her. Quickly they looked away from each other. The outer door opened and Mr. Hale came in.

"I've got the team round now," he said. "Pretty cold out there."

"I'm going to stay here awhile by myself," the county attorney suddenly announced. "You can send Frank out for me, can't you?" he asked the sheriff. "I want to go over everything. I'm not satisfied we can't do better."

Again, for one brief moment, the two women's eyes found one another.

The sheriff came up to the table.

"Did you want to see what Mrs. Peters was going to take in?"

The county attorney picked up the apron. He laughed.

"Oh, I guess they're not very dangerous things the ladies have picked out."

Mrs. Hale's hand was on the sewing basket in which the box was concealed. She felt that she ought to take her hand off the basket. She did not seem able to. He picked up one of the quilt blocks which she had piled on to cover the box. Her eyes felt like fire. She had a feeling that if he took up the basket she would snatch it from him.

But he did not take it up. With another little laugh, he turned away, saying:

"No; Mrs. Peters doesn't need supervising. For that matter, a sheriff's wife is married to the law. Ever think of it that way, Mrs. Peters?"

Mrs. Peters was standing beside the table. Mrs. Hale shot a look up at her; but she could not see her face. Mrs. Peters had turned away. When she spoke, her voice was muffled.

"Not – just that way," she said.

"Married to the law!" chuckled Mrs. Peters' husband. He moved toward the door into the front room, and said to the county attorney:

"I just want you to come in here a minute, George. We ought to take a look at these windows."

"Oh – windows," said the county attorney scoffingly.

"We'll be right out, Mr. Hale," said the sheriff to the farmer, who was still waiting by the door.

Hale went to look after the horses. The sheriff followed the county attorney into the other room. Again – for one final moment – the two women were alone in that kitchen.

Martha Hale sprang up, her hands tight together, looking at that other woman, with whom it rested. At first she could not see her eyes, for the sheriff's wife had not turned back since she turned away at that suggestion of being married to the law. But now Mrs. Hale made her turn back. Her eyes made her turn back. Slowly, unwillingly, Mrs. Peters turned her head until her eyes met the eyes of the other woman. There was a moment when they held each other in a steady, burning look in which there was no evasion or flinching. Then Martha Hale's eyes pointed the way to the basket in which was hidden the thing that would make certain the conviction of the other woman – that woman who was not there and yet who had been there with them all through that hour.

For a moment Mrs. Peters did not move. And then she did it. With a rush forward, she threw back the quilt pieces, got the box, tried to put it in her handbag. It was too big. Desperately she opened it, started to take the bird out. But there she broke – she could not touch the bird. She stood there helpless, foolish.

There was the sound of a knob turning in the inner door. Martha Hale snatched the box from the sheriff's wife, and got it in the pocket of her big coat just as the sheriff and the county attorney came back into the kitchen.

"Well, Henry," said the county attorney facetiously, "at least we found out that she was not going to quilt it. She was going to – what is it you call it, ladies?"

Mrs. Hale's hand was against the pocket of her coat.

"We call it – knot it, Mr. Henderson."