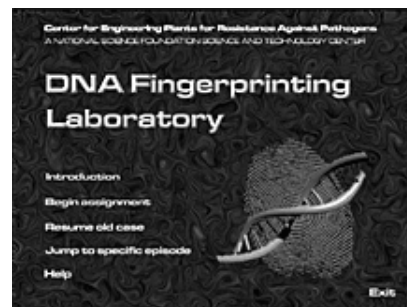




## Virtual DNA Fingerprinting Laboratory Program Outline

The *Virtual DNA Fingerprinting Laboratory 2.0* involves students in solving a forensic mystery. Over the course of seven episodes, students collect evidence, extract DNA, perform a southern blot, use PCR, and finally solve the crime. This software is designed for the high school level and above.

The game was designed to be played in a linear sequence with information building as you progress through the episodes. However, if you are interested in a specific topic, each episode may be played independently. Students may save their work and return to the game at a later time. Please see the **help & info.pdf** file located on the CD-ROM for details about installation, game play, scoring, and teacher options.



### Episodes:

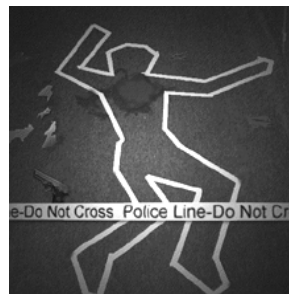
Introduction	Episode 4: Gel Electrophoresis
Episode 1: Collect Evidence	Episode 5: Southern Blotting
Episode 2: Extract DNA	Episode 6: Polymerase Chain Reaction
Episode 3: Restriction Enzymes	Episode 7: Analyze Results

### Goals:

1. Understand basic techniques and terminology in molecular biology.
2. Learn about the technique of DNA fingerprinting and its practical applications.
3. Become familiar with lab equipment and its usage.
4. Practice laboratories in virtual world so wet lab experiments will run more smoothly.
5. Follow lab protocols and safety procedures.

Following is a brief description and lab protocol for each episode. Episode goals, articles, quizzes, and video segments are also listed.

### Introduction



Police are called to investigate an alarm sounded at Newgene Technology Inc. Upon your arrival, a broken window was discovered, as well as the body of the CEO of the company, I.M. Jeanus. The crime scene was immediately secured and you and your forensic team were called in to collect evidence for DNA testing.

Police investigators have interviewed witnesses and viewed security tapes to come up with four suspects. Each of these four suspects will have blood drawn so their DNA fingerprint can be compared to DNA from the evidence samples you will be testing.



### Topics Covered

Introduction to DNA fingerprinting  
Overview of RFLP (Restriction Fragment Length Polymorphism)  
Overview of PCR (Polymerase Chain Reaction)

## Episode 1: Collect Evidence

Now that you have examined the crime scene and recovered all physical evidence left at the site, lab work will begin in earnest. First, read the articles on your office computer to get up to speed on DNA testing. Also, review the suspect information in your desk drawer that has been gathered by police detectives. These files will be updated as more information is obtained. Next, go to the evidence room to determine what items are suitable for DNA analysis.



### Topics Covered

DNA structure and function  
Materials appropriate for DNA extraction  
Laboratory safety

### Lab Protocol

1. Review computer articles on collecting DNA evidence.
2. Examine suspect files.
3. Obtain proper protective gear to avoid contaminating the samples.
4. In the evidence room, select three appropriate samples from materials collected at the scene.

### Articles

Obtaining DNA Evidence  
DNA Fingerprinting  
DNA Structure and Function

### Video *Windows version only*

Introduction — Barbara Soots, Education Coordinator, CEPRAP

## Part 2: DNA Extraction

You are now ready to extract DNA from the evidence. This is the first step in both RFLP and PCR procedures. While you are extracting DNA from the blood sample, your assistants will complete similar extraction procedures for the skin cells and hair follicles. Their results will be available as you require them.



### Topics Covered

DNA extraction technique  
Practical applications of DNA fingerprinting  
Lab equipment: centrifuge, waterbath  
Laboratory safety

### Lab Protocol

1. Centrifuge blood sample.
2. Pour off excess liquid, leaving a cell pellet.
3. Add lysis buffer and spin in centrifuge. Pour off excess liquid.
4. Add protein digest solution.
5. Incubate in a 45 degree water bath.
6. Add NaCl solution.
7. Add ethanol and gently mix so the DNA will come out of the solution.

### Articles

Extraction from Human Material  
DNA Extraction  
Recent cases of DNA Fingerprinting

### Video *Windows version only*

DNA Extraction – *George Bruening, Director, CEPRAP*

## Part 3: Restriction Enzymes

Now that you have extracted DNA from the evidence, you are ready to begin the RFLP method to analyze DNA from the blood cells. DNA from the skin and hair cells will be analyzed at a later time using the PCR procedure. The first step in the RFLP method requires that the DNA be cut into fragments. Molecular scissors called restriction enzymes are used because they recognize and cleave specific sequences on the DNA strands. Take the blood DNA sample you extracted in your last assignment and follow the steps as outlined in your lab manual.



### Topics Covered

Restriction enzymes: origin, function, and naming  
Micropipet technique  
Laboratory safety

### Lab Protocol

1. Obtain ice and restriction enzyme from freezer.
2. Get buffer solution from freezer.
3. Set a micropipet to 5  $\mu\text{l}$ .
4. Put a tip on the micropipet and obtain 5  $\mu\text{l}$  of restriction buffer. Dispense into an empty microtube.
5. Add 4  $\mu\text{l}$  of DNA to the tube with buffer. Be sure to put on a new micropipet tip.
6. Add 2  $\mu\text{l}$  of restriction enzyme to the tube.
7. Incubate tube at 37  $^{\circ}\text{C}$ .

### Articles & Quizzes

Enzyme Naming  
Restriction Enzymes  
Enzyme Quiz (30 points)

### Video *Windows version only*

Restriction Enzymes – *Adriana Bernal, Researcher, CEPRAP*

## Part 4: Gel Electrophoresis

As part of the RFLP technique, the DNA fragments you have cut with restriction enzymes need to be separated. We will utilize gel electrophoresis to accomplish this task. Be sure to follow the gel electrophoresis protocol outlined in the lab manual.



### Topics Covered

Explanation of gel electrophoresis  
Loading DNA samples into a gel box  
Buffer solutions  
Dilution calculations  
Use of lab equipment: balance, micropipet, gel box, power supply  
Laboratory safety

## Lab Protocol

1. Make the TBE (Tris Borate EDTA) buffer solution required for this experiment in the chem room.
  - a) Measure 2.7 grams of Tris Base on a balance, and add to a beaker.
  - b) Measure 1.4 grams of Boric Acid, and add to the same beaker.
  - c) Measure 0.2 grams of EDTA, and add to the same beaker.
  - d) Using a graduated cylinder, add 250 ml of deionized water to the beaker.
2. Get a pre-poured agarose gel from the storage container in the chem room refrigerator.
3. Obtain a 20  $\mu$ l micropipettor.
4. Place gel in gel electrophoresis box.
5. Add 250 ml of 1X TBE buffer to gel box.
6. Put a new tip on the micropipet and add 2  $\mu$ l of loading dye to your DNA sample.
7. Put a new tip on the micropipettor and add 10  $\mu$ l of DNA with loading dye to the well in your gel.
8. Put the lid on the gel box and hook up red and black leads to the power supply.
9. Turn the power on, set the meter to 100 volts, and run for 90 minutes.

### Articles & Quizzes

Gel Electrophoresis Technique  
Applications of Gel Electrophoresis  
Buffer Preparation  
Dilutions Review  
Dilutions Quiz (20 points)

### Video *Windows version only*

Gel Electrophoresis – Kevin Fort, Researcher, CEPRAP

## Part 5: Southern Blot

To complete the RFLP technique you must now blot and probe the DNA that you separated by gel electrophoresis. We will analyze the results later. Check your computer files for more information on Southern blotting and on DNA probes. Follow the protocol outlined in the lab manual to isolate the specific DNA bands that we need to investigate.



### Topics covered

DNA probes  
Procedure for performing a southern blot  
DNA fingerprinting applications  
Lab equipment: hybridization oven, darkroom  
Laboratory safety

### Lab Protocol

1. In the hot room, place agarose gel from the blood sample into a tray.
2. Add denaturing buffer solution to tray.
3. Obtain a sheet of nylon membrane and place on the gel in the tray.
4. Obtain a stack of paper towels and add to top of gel. Wait 8 hours for the transfer of DNA to be complete.
5. Bake the nylon membrane in the hybridization oven for 2 hours. Membrane is then placed in a tray with new buffer solution.
6. Add the radioactive probe to the tray and incubate for 6 hours.
7. Go to the darkroom and place radioactive membrane in metal film cartridge.
8. Turn off room lights and turn on red light. Next, get a piece of x-ray film.
9. Place film on top of membrane. Close cartridge. Film results will be analyzed later.

## Articles & Quizzes

RFLP

Probe Information and Examples

Understanding Gene Testing

Paternity Testing

## Video *Windows version only*

Southern Blot – Jafar Yaghoobi, Researcher, CEPRAP

## Part 6: Polymerase Chain Reaction

Now you are ready to analyze the DNA from the hair and skins samples by using the Polymerase Chain Reaction (PCR) procedure. Because the amount of DNA originally extracted from the hair and skin samples was very low, there was not enough DNA to use the RFLP technique. Instead, you will amplify the DNA samples by using PCR, and then run the results on a gel. The results will be analyzed later.

### Topics covered

PCR explanation and technique

Taq polymerase and primers

Lab equipment: thermocycler, micropipet

Laboratory safety



### Lab Protocol

1. Take DNA tube to the thermocycler in the chem room.
2. Obtain a micropipettor and put on a sterile tip.
3. Use the micropipet to add the PCR mixture with primers to tube with DNA.
4. After adding a new sterile tip, add the Taq polymerase enzyme to the DNA tube.
5. Put DNA tube with PCR mixture into thermocycler.
6. Program the thermocycler to run the following sequence:
  - a) 1 minute at 94 °C
  - b) 1 minute at 65 °C
  - c) 1 minute at 72 °C
  - d) repeat the above steps 29 times

## Articles

PCR Technique

PCR vs RFLP

PCR Diagram

## Video *Windows version only*

PCR– Rob Haworth, Researcher, CEPRAP & teacher, Laguna Creek HS

## Part 7: Analyze Results

All tests are complete. In addition to the results from the blood, skin and hair evidence collected at the crime scene, you also have test results from the DNA analysis that you performed on the victim and three suspects. Go to the darkroom to analyze the test results. Compare the results with the DNA of the suspects and victims individuals to determine the guilty party.



### Topics covered

How to read RFLP and PCR test results  
Loci targeted for DNA fingerprinting  
DNA fingerprinting in court cases

### Lab Protocol

1. Obtain test results from filing cabinet.
2. Take results to darkroom lightbox for analysis.
3. One at a time, put results from each type of sample on the lightbox to compare bands and find the guilty suspect.

### Articles

Analyzing Test Results & Examples  
D1S80 Locus  
DNA Fingerprinting in Court

### Video

Conclusion – *Barbara Soots, Education Coordinator, CEPRAP*

The Center for Engineering Plants for Resistance Against Pathogens is a National Science Foundation Science and Technology Center located at the University of California, Davis.

CEPRAP's *Virtual DNA Fingerprinting Laboratory 2.0* is copyrighted by the Regents of the University of California. The software is intended to provide a general introduction to the topic of DNA fingerprinting. Please note that some of the protocols have been simplified to lend themselves to this format.

This is a work of fiction. Resemblance to any persons living or dead is purely coincidental. Any opinions, findings, conclusions, or recommendations are those of the authors and do not necessarily reflect the views of the NSF.

This program is distributed free of charge to educators and may be installed on multiple computers for classroom use.

Please visit our web site at <http://ceprap.ucdavis.edu/Outreach/vdna.htm> to receive program upgrades and fill out a software evaluation.

If you have questions or comments regarding this software or CEPRAP's other educational programs, please contact:

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