Biotechnology Basics™ by Ellyn Daugherty

Crime Scene DNA Analysis Using RFLP Technology

(Cat. # BBED-4M)

Developed in partnership with

Crime Scene DNA Analysis Using RFLP Technology

Teacher's Guide

The following laboratory activity is adapted from labs in Biotechnology: Science for the New Millennium by Ellyn Daugherty and G-Biosciences' DNA Fingerprinting Lab (BE-104).. For more information about *Biotechnology: Laboratory Manual* curriculum, please visit www.emcp.com/biotechnology. This kit is produced under license from Paradigm Publishing, Inc., a division of New Mountain Learning.

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About Ellyn Daugherty: Ellyn Daugherty is a veteran biotechnology educator and recipient of the Biotechnology Institute's National Biotechnology Teacher-Leader Award. She is the founder of the San Mateo Biotechnology Career Pathway (SMBCP). Started in 1993, SMBCP has instructed more than 10,000 high school and adult students. Annually, 30-40 SMBCP students complete internships with mentors at local biotechnology facilities.

About G-Biosciences: In addition to the Biotechnology by Ellyn Daugherty laboratory kit line and recognizing the significance and challenges of life sciences education, G-Biosciences has initiated the BioScience Excellence™ program. The program features hands-on teaching kits based on inquiry and curiosity that explore the fundamentals of life sciences and relate the techniques to the real world around us. The BioScience Excellence™ teaching tools will capture the imagination of young minds and deepen their understanding of various principles and techniques in biotechnology and improve their understanding of various social and ethical issues.

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MATERIALS INCLUDED

This kit has enough materials and reagents for 8 lab groups (16 student pairs or 32 students in 4/groups).

- 8 tubes of Victim DNA, 20µl
- 8 tubes of Suspect 1 DNA, 20µl
- 8 tubes of Suspect 2 DNA, 20µl
- 8 tubes of Crime Scene 1 DNA, 20µl
- 8 tubes of Crime Scene 2 DNA, 20µl
- 8 tubes of DNA Loading Dye Buffer, 6X, 50µl
- 8 tubes of of DNA Loading Buffer [6X] for practice loading, 350 µL
- 8 tubes Cleaving Enzyme Mix (Restriction Enzyme Mix), 120µl
- 1 tube Sterile Water
- 1 bottle of 0.8% agarose in 1X TAE
- 1 bottle TAE Buffer (50X), 40ml
- 1 vial LabSafe™ Nucleic Acid Stain

ADDITIONAL EQUIPMENT & MATERIALS REQUIRED

- Horizontal Gel Box with either 6-well or 8-well combs, with a poured agarose gel covered with 1X TAE buffer
- Power Supply, 300V
- P-20 Micropipette* and tips*, 8
- Permanent lab marker, 8
- "TRASH" beaker or container, 8
- Microwave
- 37°C Hot water bath or incubator
- UV Transilluminator/UV light box/ Blue LED box
- Deionized or distilled water, 4L

OPTIONAL MATERIALS

- P20 micropipette and tips
  These can be purchased separately

Cat. #	Fisher Sci Cat #	
BT1505	501059654	Pipette, Variable, P20

SPECIAL HANDLING INSTRUCTIONS

- Store DNA, LabSafe™ Nucleic Acid Stain and Cleaving Enzyme Mix at -20°C.
- All components can be stored at room temperature until ready to use.
- Use paper towels to wipe spills when using the electrophoresis equipment.

GENERAL SAFETY PRECAUTIONS

- The reagents and components supplied in the Biotechnology Basics™ by Ellyn Daugherty kits are considered non-toxic and are safe to handle (unless otherwise noted), however good laboratory procedures should be used always. This includes wearing lab coats, gloves and safety goggles.

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- The teacher should 1) be familiar with safety practices and regulations in his/her school (district and state) and 2) know what needs to be treated as hazardous waste and how to properly dispose of non-hazardous chemicals or biological material.
- Students should know where all emergency equipment (safety shower, eyewash station, fire extinguisher, fire blanket, first aid kit etc.) is located and be versed in general lab safety.
- Remind students to read all instructions including Safety Data Sheets (SDSs) before starting the lab activities. A link for SDSs for chemicals in this kit is posted at www.gbiosciences.com
- At the end of the lab, all laboratory bench tops should be wiped down with a 10% bleach solution or disinfectant to ensure cleanliness. Remind students to wash their hands thoroughly with soap and water before leaving the laboratory. All used culture vials, strips, tips, or may be discarded in regular trash.

TEACHER'S PRE EXPERIMENT SET UP

1. Set up a gel box (with either an 8-well, 9-well, or 10-well comb) and a power supply at 4 stations surrounding a spot where the teacher can stand and instruct. In Part I, four teams of students will surround each gel box and follow the teacher's directions.

2. Prepare the agarose for gel pouring gel as follows:

    - Loosen the cap on the bottle of prepared agarose, so they vent steam and pressure when heating. The agarose gets very hot, very quickly and can cause severe burns. Wear protective goggles when preparing agarose solution. Use heat-protective gloves or "hot hand" mitts when handling hot agarose solutions. Do not use latex gloves.

    - Heat solution in a microwave on 50% power for about 3-4 minutes. Keeping the container away from your face, check to see that all the agarose has dissolved completely and that no crystals or lumps of agarose are left unmelted. Heat for 0.5-1-minute intervals, if necessary, until agarose has dissolved.

    - Allow the agarose solution to cool to about 60°C. 60°C is still pretty hot but it is cool enough to hold for pouring. While it cools, orient the gel electrophoresis trays into position for casting (pouring) the gel(s). Some gel trays use tape on the ends, some use gates or buffer dams, and some are wedged into the gel box for pouring. Please refer to the manufacturer's instructions for how to set up and use your gel box.

    - Once the agarose has cooled to the point it can be held comfortably in one's hand (around 60°C), add 50µl of the LabSafe™ Nucleic Acid Stain to the warm agarose. Swirl gently to mix.

3. Pour a 7-mm thick gel in each gel tray **making sure that a gel comb is placed both at the top and in the center slot of the gel tray**. Depending on the comb, the result should be 2 rows of 6-8 wells across the top and across the center of the gel.

    - Quickly, pour the agarose into one or more gel casting trays, depending on your electrophoresis equipment, to about 7mm thick. Using an appropriate size gel-well comb (8-, 9- or 10-well comb), place the comb in the center slots of the hot agarose tray to create wells that will hold at least 25 µl. Make sure that the comb is level so that all the wells will be of the same dimensions.

- Let the gel(s) solidify, 30-40 minutes depending on the size of each gel. This is longer than "usual" to make sure the gels are firm. Since the gels will be used during Part II (maybe on the 2nd day) of the lab activity after Part I, place the gel, still in its tray with the comb still in the gel, into a weigh boat, and add enough 1X TAE buffer to keep it damp until ready to use. Slide the weigh boat with the gel into a quart-sized Ziploc® bag. Seal it and keep in a cool, dark place for up to 24 hours or in a refrigerator for several days. Do not allow the gels to freeze.

- Before students do Procedure Part II, gently lower a tray with the pre-cast gel with the comb in it into the gel box filled with 1X TAE buffer. Make sure the top wells are oriented on the negative (black) electrode end of the gel box. Hold the ends of the gel and tray as it lowers into the buffer so that it settles straight down and doesn't break.

4. At each of the four gel box set-ups, 2 lab groups will share a
    a. Horizontal Gel Box with either 6-well or 8-well combs, with a poured agarose gel covered with 1X TAE buffer
    b. Power Supply, 300V

5. At a common use area:
    a. 37°C Hot water bath or incubator
    b. UV Transilluminator/UV light box/ Blue LED box
    c. Deionized or distilled water, 4L

6. Distribute to each group the following components from the kit:
    - 1 tube of 20µl Victim DNA
    - 1 tube of 20µl Suspect 1 DNA
    - 1 tube of 20µl Suspect 2 DNA
    - 1 tube of 20µl Crime Scene 1 DNA
    - 1 tube of 20µl Crime Scene 2 DNA
    - 1 tube of 120µl Restriction (Cleaving) Enzyme Mix in Reaction Buffer
    - 1 tube of 50µl DNA Loading Dye Buffer
    - 1 tube of 200 µL of Practice DNA Loading Dye
    - P-20 micropipette and tips
    - Permanent lab marker
    - TRASH" beaker or container

TIME REQUIRED

- 1-hour for teacher preparation of gels and distribution of reagents
- 1-hour lab period for restriction digestion of DNA samples
- 1-2-hour lab period for loading and running samples on the gel and analyzing gels

NOTE: *If necessary, the gels can be stored for up to 5 days after gel electrophoresis of the samples. Place the gels in a Ziploc bag with a small amount of running buffer and keep in a fridge. Optimal results are achieved when viewed immediately, storage may result in some diffusion of the bands, but the result is still easy to see.*

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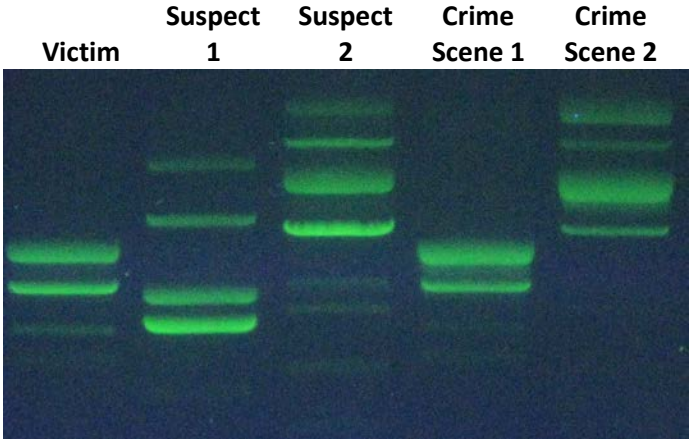
NEXT GENERATION SCIENCE STANDARDS ADDRESSED

- HS-LS1: From Molecules to Organisms: Structures and Processes
- LS1.A: Structure and Function

For more information about Next Generation Science Standards, visit: http://www.nextgenscience.org/

EXPECTED RESULTS



Reflection/Analysis

Describe your results. Does either suspect have a DNA fingerprint (RFLP banding pattern) that matches either of the DNA sample evidence from the crime scene? Explain. What do you conclude from the samples tested and the data collected?

The banding pattern of the victim's DNA matches the banding pattern of the crime scene DNA evidence #1. The banding pattern of the Suspect #2 DNA matches the banding pattern of the crime scene DNA evidence #2. The banding pattern of the Suspect #1 DNA does not match the banding pattern of any other of the DNA samples.

Evidence 2 collected at the crime scene identifies Suspect 2 as being at the crime scene; Evidence 1 belonged to the victim. The results show that Suspect 2 was present at the crime scene, whereas no DNA for suspect 1 was found at the crime scene, implying that the suspect was not there.

Thinking Like a Biotechnician:

1. Is there any way two people could have the same DNA fingerprinting result.

Answer: If they are identical twins they should have the same RFLP banding pattern (DNA fingerprint).

2. Discuss ways that someone's DNA could be at the scene of a crime without being the victim or suspect.

Answer: Answers may vary but possible answers could include contamination of the scene, planting of evidence, contamination of samples after collection, etc.

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OBJECTIVES

Can restriction digestion enzymes produce DNA fragments that are recognizable on an electrophoresis gel?

Using restriction enzyme digestion and DNA banding fingerprints can a suspect be recognized or excluded from a crime scene investigation?

BACKGROUND

Each organism contains a unique DNA genetic code of the nitrogen-containing bases adenine, cytosine, guanine, and thymine (A, C, G, T). The genetic code is deciphered and into all of the proteins made by an organism

Scientists have methods to determine the differences in the genetic code of different individuals. The DNA data is called a **DNA fingerprint** and it may be used to identify individuals.

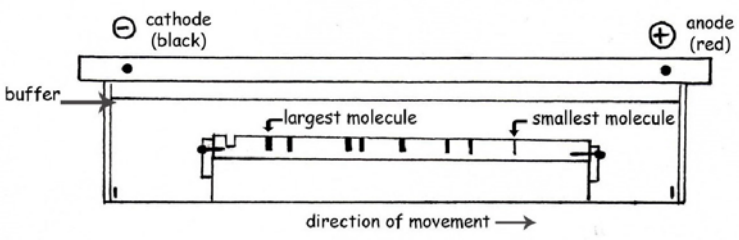
In DNA fingerprinting, DNA is isolated from cells in a sample. Relatively short pieces of the long strands of DNA are produced and, because the order of the A, C, G, and Ts are different from individual to individual, the fragments are of different lengths. The pieces of DNA are run and separated on an agarose gel using electrophoresis. The DNA fragments separate on the gel based on their length. The pattern of DNA fragments on a gel will be unique to an individual and is recognized as a DNA fingerprint.

When a crime is committed, often a sample of cells (maybe from blood, skin, body fluids, or hair follicles) is left at the scene. DNA can be extracted from the sample cells as well as any suspect's cells and DNA fingerprinting techniques can be used to distinguish one person's DNA from another.

Two common methods of DNA fingerprinting are RFLP analysis and Polymerase Chain Reaction. In this lab activity, RFLP analysis is used. In RFLP analysis, sections of DNA that are known to differ in individuals are subjected to **restriction enzyme digestion** and gel electrophoresis analysis.

Restriction enzymes are proteins that are used to recognize and cut across specific A, C, G, and T sequences within DNA molecules. More than 4000 restriction enzymes recognizing over 300 different sequences have been discovered and isolated from different bacterial species. Restriction enzymes are very specific and will only cut DNA at certain A, C, G, and T sequences which results in different fragments in different individuals. The resulting pieces are called **restriction fragments.** Restriction fragments are run on a gel, banding out at certain spots because of their size.

Gel electrophoresis is one of several ways that molecules are studied in labs. The technique uses a gel and an electric field to separate molecules based on size, shape, and/or charge. Most frequently, samples of nucleic acids (DNA and RNA) and proteins are analyzed using gel electrophoresis. In this activity, the DNA restriction fragments will be run on a gel.



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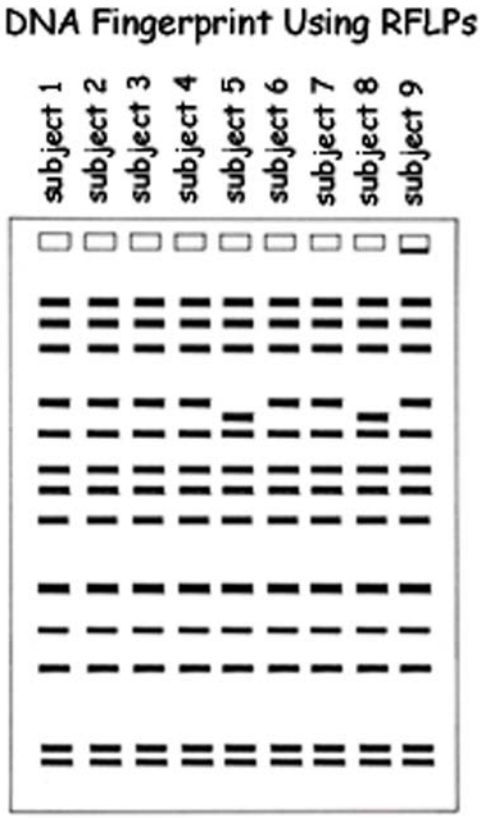
DNA molecules in solution have a net negative charge due to their phosphate groups. Because of the negative charge, DNA molecules are attracted to the positive end of a "running" gel box. The agarose gel material acts as a molecular sieve separating longer DNA fragments from shorter ones, thus separating the molecules based on their size and when stained are visible as a DNA fingerprint-banding pattern.

Differences in the gel-banding pattern of restriction fragments from different samples are called **restriction fragment length polymorphisms** (RFLPs). Similarities and differences in RFLPs and thus individuals' DNA are shown in the unique gel-banding pattern. RFLP technologies have been used for four decades.



All samples show the same restriction digestion pattern after being cut by a single restriction enzyme except subjects 5 and 8. This indicates a difference in sequence in these individuals due to a mutation.

In recent years, Polymerase Chain Reaction (PCR) technology has been used for DNA fingerprinting. The PCR process targets specific sections of genetic information and reproduces it millions of times. Variations in the DNA information can be deduced by how pieces are targeted and duplicated. Since PCR requires additional equipment and reagents, in this lab activity, RFLP analysis is used to produce DNA fingerprints.

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For this investigation, DNA samples have been collected from the scene of an assault and battery crime. DNA has been isolated from samples from:

- The victim blood
- Crime scene sample = blood found on the victim's clothing
- Crime scene sample = hair with hair follicle found on the victim's clothing
- Suspect #1 blood
- Suspect #2 blood

The DNA samples have been sent to your lab. Using restriction enzyme digestion and electrophoresis, can DNA banding fingerprints recognize or exclude one of the suspects?

MATERIALS FOR EACH GROUP

Each group should have the following components from the kit:

- 1 tube of 20µl Victim DNA
- 1 tube of 20µl Suspect 1 DNA
- 1 tube of 20µl Suspect 2 DNA
- 1 tube of 20µl Crime Scene 1 DNA
- 1 tube of 20µl Crime Scene 2 DNA
- 1 tube of 120µl Restriction (Cleaving) Enzyme Mix in Reaction Buffer, * Instructor prepared
- 1 tube of 50µl DNA Loading Dye Buffer
- 1 tube of 200 µL of Practice DNA Loading Dye
- P-20 micropipette and tips
- Permanent lab marker
- TRASH" beaker or container

Not supplied in kit:

At each of the four gel box set-ups, 2 lab groups will share a

1. Horizontal Gel Box with either an 6-well or 8-well combs, with a poured agarose gel covered with 1X TAE buffer
2. Power Supply, 300V

At a common use area:

1. 37°C Hot water bath or incubator
2. UV Transilluminator/UV light box/ Blue LED box
3. Deionized or distilled water, 4L

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PROCEDURE

Part I Prepare the Restriction Digestion of DNA Samples

1. Label five each of the DNA sample tubes with your groups' ID. Make sure that the DNA in each tube is at the bottom of the tube. Centrifuge or wrist-flick the DNA to the bottom of the tube before going to Step 2. All the DNA tubes should have the sample volume in them.

2. Using a micropipette, transfer 20µl of the restriction enzyme mix into each of the labeled DNA tubes. Make sure you watch the restriction enzyme sample mix into the DNA sample at the bottom of the tube. Change tips for each transfer of enzyme to avoid cross-contamination.

3. Tightly cap each tube and place all five tubes in a water bath or incubator at 37°C for 15-30 minutes. The incubations can be left at 37°C overnight. Use immediately or store on ice or in a refrigerator.

Part II Gel Electrophoresis of RFLPs

1. Pool all the samples to the bottom of their tubes by wrist-flicking or centrifugation.

2. Using a micropipette, add 9µl of DNA Loading Dye Buffer to each tube. Change tips for each transfer of loading dye to avoid cross-contamination.

3. An agarose gel with 2 rows of sample wells (one for each lab group) should have been prepared by your teacher/supervisor. Each lab group will have the top or the middle row of wells. Each student takes turns practice loading samples into the sample wells of a gel.

    a. The agarose gel slab should be sitting on a gel tray so that the ends of the gel are facing the electrode ends of the box. There should be enough electrophoresis buffer in the gel box to cover the gel by about 10 cm since too much buffer slows down the electrophoresis.

    b. The wells are numbered, starting at 1, from bottom to top, when the negative electrode is on your left side and the positive electrode is on your right side.

    c. The instructor will demonstrate how to use a micropipette to load a sample into a gel well. Practice loading 20 µL (or so) of practice loading dye into any well #6. Be careful to never put a loading tip into a well. Instead release the sample to be loaded just above the well and the sample will sink down into the well.

    d. Remove the practice samples by rinsing the wells using the buffer in the gel box. Have each partner practice loading by repeating step 3c and d.

4. Make sure the gel box is situated where it will be loaded and run. Once samples are loaded the gel should not be moved.

5. Using a micropipette, students in the lab group should take turns loading samples into the corresponding well as directed in the table below. Change micropipette tips for each sample that is loaded.

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DNA Fingerprinting Crime Scene Investigation Gel Loading Chart

Well 1	Well 2	Well 3	Well 4	Well 5
Victim DNA	Suspect 1 DNA	Suspect 2 DNA	Crime Scene 1 DNA	Crime Scene 2 DNA

6. Turn on the power supply and turn the voltage to 115 volts. Check to see if current is running through the gel box. Record the current. _____mA. There should be at least 30 mA running through the gel box.

7. Run the electrophoresis for 15-20 minutes or until you can see that the dye molecules in samples have moved approximately ¾ of the way towards the positive (red) electrode.

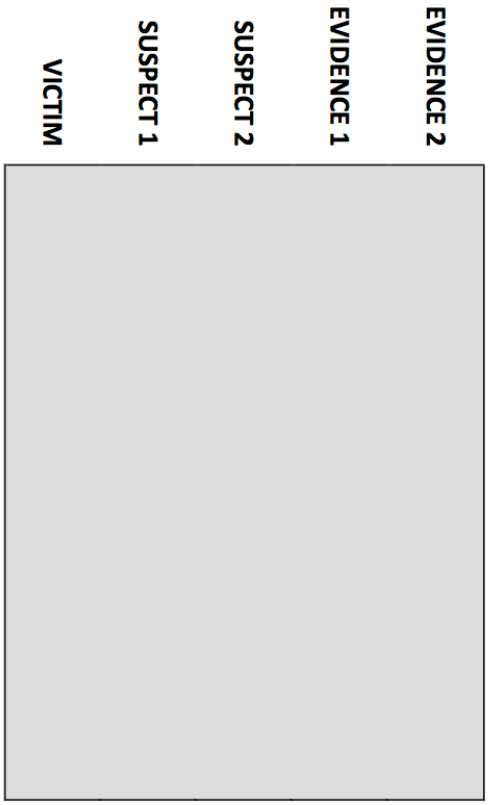
Part III Analysis of DNA Fingerprints

1. Turn off the power supply and remove the cover of the gel box. Holding the tray at each end, so the gel doesn't slide off, move the gel tray over to the UV light box/ Blue LED box for DNA band visualization. Gently slide the gel onto the surface of the light box. Your instructor will illuminate the gel.
   CAUTION: Unless directed by your instructor otherwise, wear UV Safety Goggles when working around the UV light box/ Blue LED box.

2. By turning on the UV transilluminator, you should be able to see the DNA restriction fragment bands in all lanes of samples. RFLP bands in different lanes of the gel that match can be assumed to originate from the same person.

3. Draw a representation of the bands visualized on the gel:

4. The illuminated gel can be photographed for a permanent record.



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REFLECTION/ANALYSIS

Describe your results. Does either suspect have a DNA fingerprint (RFLP banding pattern) that matches either of the DNA sample evidence from the crime scene? Explain. What do you conclude from the samples tested and the data collected?

Thinking Like a Biotechnician

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