INVESTIGATION 9

BIOTECHNOLOGY: RESTRICTION ENZYME ANALYSIS OF DNA*

How can we use genetic information to identify and profile individuals?

THE SCENARIO

"OMG! Is that blood?" Laurel nearly broke Marcus's arm as she tried to push past him into the classroom.

Marcus grabbed the sleeve of her cardigan and yanked her back. "Don't! Can't you see the glass?" Laurel tried knocking his hand free, but the 6'4" varsity basketball captain held tight. He made her settle for looking from under his armpit.

Not that what she saw would make any sense. Their AP Biology lab looked like a riot scene. Four chairs and a potted plant were overturned in the center of the room, and broken pieces of glass were scattered across the floor along with several wet red drops. Plink … plink … plink. *Marcus's eyes were drawn to the teacher's desk where droplets of brownish liquid fell from a paper cup and collected in a puddle on the linoleum.*

"What happened?" Laurel asked. "Did somebody get hurt?" Laurel and her classmates had gathered in front of the door and strained to see inside Room 102.

Marcus inspected the scene and raised his right arm above his head, his fingers spread apart as if taking a shot from the free throw line. "Stay back!"

"Where's Ms. Mason?" Laurel said. "She told me I could meet her before class to review for the quiz."

"Okay, folks, keep it down." Mr. Gladson, the teacher in the classroom next door, came into the hall. His white lab coat was streaked with several rust-colored stains. The pungent odor of formaldehyde permeated the corridor. "In case you haven't noticed, the bell has rung." He wiped his nose with a tissue and then tossed it into a nearby trash can. A girl's fake shriek from inside the anatomy lab rose above the buzz of Marcus's classmates.

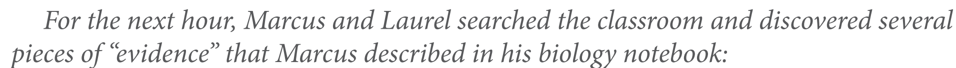
"What's going on?" Bobby's high-pitched whine was unmistakable—and so was the scent of his bubble gum.

"I think something might've happened to Ms. Mason," Marcus said. He dug around in his backpack and pulled out a magnifying glass. "We've got a crime scene to process."

"Go figure," Laurel said. "Sherlock Holmes in a varsity jacket."

---

* Transitioned from the *AP Biology Lab Manual* (2001)



For the next hour, Marcus and Laurel searched the classroom and discovered several pieces of "evidence" that Marcus described in his biology notebook:

- Ten small drops on floor confirmed by Kastle-Meyer test to be blood
- Shard of glass from a broken 500-mL Erlenmeyer flask, edge smeared with a reddish stain
- Paper cup with lipstick stains, presumed to be Ms. Mason's, found on her desk
- Wad of bubble gum stuck underneath overturned chair
- Mr. Gladson's discarded tissue recovered from trash can in hall outside Room 102
- Bobby's test on photosynthesis with large "F" scrawled in red ink on first page
- Copy of email from Mr. Gladson to Ms. Mason asking her to give up position as department chair

Marcus's new game was afoot!

BACKGROUND

Applications of DNA profiling extend beyond what we see on television crime shows. Are you sure that the hamburger you recently ate in the local fast-food restaurant was actually pure beef? DNA typing has revealed that often "hamburger" meat is a mixture of pork and other nonbeef meats, and some fast-food chains admit to adding soybeans to their "meat" products as protein fillers. In addition to confirming what you ate for lunch, DNA technology can be used to determine paternity, diagnose an inherited illness, and solve historical mysteries, such as the identity of the formerly anonymous individual buried at the Tomb of the Unknown Soldier in Washington, D.C.

DNA testing also makes it possible to profile ourselves genetically — which raises questions, including *Who owns your DNA and the information it carries?* This is not just a hypothetical question. The fate of dozens of companies, hundreds of patents, and billions of dollars' worth of research and development money depend on the answer. Not only does this investigation provide an opportunity for students to learn and apply techniques used by scientists in creating genetic profiles, it raises questions that students can explore about the ethical, social, and medical issues surrounding the manipulation of genetic information.

In this investigation, students begin by familiarizing themselves with the procedure *without running an actual gel electrophoresis*. Then students will use restriction endonucleases and gel electrophoresis to analyze DNA sequences by creating genetic "fingerprints." Students will apply mathematical routines to determine the approximate sizes of DNA fragments produced by restriction enzymes to solve the forensic mystery presented in the scenario. There are two parallel activities going on: conducting an investigation requiring technical skills and identifying likely suspects in the disappearance of the teacher. By learning and applying these fundamental skills, students acquire the tools to conduct more sophisticated biotechnology investigations.

PREPARATION

Materials and Equipment

Supplies for this investigation can be purchased through several commercial vendors. A partial list of suppliers is provided in the Supplemental Resources section.

The materials described below are included in an eight-station kit and are sufficient for eight complete setups of the investigation. You can also purchase a less expensive four-station kit to accommodate smaller class sizes or pool leftover supplies.

A list of materials and supplies needed for each student work station is supplied in the student version of this investigation. Students are encouraged to set up their own work-stations. **Note:** Materials and instructions in the student version of this investigation are more generic to accommodate different vendors' products.

Materials Included in Kit (Eight-Station)

- 20 μL vials of lambda DNA
- 20 μL vials of lambda DNA cut with *Eco*RI
- 20 μL vials of lambda DNA cut with *Hin*dIII
- Optional: samples of lambda DNA cut with other restrictions enzymes (see Supplemental Resources) to make "evidence" samples
- TAE buffer 50x concentrate
- Agarose
- Disposable plastic needle-nose transfer pipettes
- Methylene blue gel and buffer stain
- Staining trays
- Disposable gloves
- Semi-log graph paper and rulers
- Dry lab activity sheets

Note: The DNA is often stabilized for storage at room temperature. However, if the DNA is to be kept for more than six weeks, it should be frozen or refrigerated. All other components may be stored at room temperature.

Materials Needed but Not Supplied

- Gel electrophoresis chambers and power supplies
- Masking tape (if needed to seal gel casting trays)
- Racks for holding samples
- 1-L graduated cylinder for diluting and measuring TAE buffer
- Container for holding 5 L of 1x TAE buffer
- 60°C water bath for keeping agarose liquid until poured into casting tray
- Microwave oven OR hot plate with stir bar OR boiling water bath for melting agarose
- Permanent markers
- Distilled or deionized water
- Aluminum foil

Optional Equipment

- Microcentrifuge for pooling DNA (*or* samples can be tapped to pool)
- White-light box or overhead projector for viewing stained gels (Note: Most stained DNA fragments can be seen with the naked eye.)
- Plastic bag or container with lid if gels need to be stored overnight

Preparation of DNA "Evidence" Samples

At minimum, you will have to prepare DNA "evidence" found at the mock crime scene from the blood spattered on the floor — Ms. Mason, Mr. Gladson, and Bobby. You might want to add other names to the list of suspects. (An alternative is to have a team of students create a mock crime scenario for other teams, with you providing appropriate "evidence" samples of DNA.) You will have to reserve a vial of lambda DNA cut with *Hin*dIII to serve as a marker, providing a set of RFLPs of known sizes (standard).

To make these evidence samples, you can use the vials of lambda DNA cut with *Eco*RI and *Hin*dIII and the "uncut" samples of lambda DNA provided in the kit. Remove the labels from the vials and replace them with new labels indicating the source of the sample, e.g., "Suspect 1," "Suspect 2," "Blood," or "Ms. Mason," "Mr. Gladson," "Bobby," "Principal." You can purchase samples of lambda DNA cut with other restriction enzymes from commercial vendors, or you can combine a sample of lambda DNA cut with *Eco*RI with a sample cut with *Hin*dIII to mix things up. To be more tricky, give each student group DNA "evidence" that leads to different perpetrators, i.e., for one group, Bobby is guilty, whereas for another group, Mr. Gladson is the likely culprit. Since there is no single correct answer for "whodunit," you can take the scenario in multiple directions.

Pool Small Volumes of DNA

During shipping, the small volume of DNA in each tube may become spread as a film around the storage tube wall or cap. Therefore, you should pool the DNA solutions at the bottom of their storage tubes by using one of the following methods:

1. Spin the tubes briefly in a microcentrifuge.
2. Spin the tubes briefly in a preparatory centrifuge, using adapter collars for 1.5 mL tubes.
3. Tap the base of the tubes sharply on the bench top.

Note: Methylene blue stain stain has been added to the DNA samples for better visualization.

Preparation of TAE Buffer

Because tris-acetate-EDTA (TAE) buffer solution is stable, it can be made ahead of time and stored in a carboy or other container in the refrigerator until you are ready to use it.

For the eight-station kit: To make 1x buffer from the 50x stock, mix 100 mL of 50x TAE concentrate with 4,900 mL of distilled or deionized water. Mix for 1–2 minutes. This is a 1:50 dilution for a final volume of 5 L.

For the four-station kit: To make 1x buffer from the 50x stock, mix 50 mL of 50x TAE concentrate with 2,450 mL of distilled or deionized water. Mix for 1–2 minutes. This is a 1:50 dilution for a final volume of 2.5 L.

Preparation of Agarose Solution/Casting Gels

Prepare 0.8% agarose solution before class on Lab Day 1. You will use approximately 50 mL of agarose per gel depending upon your electrophoresis apparatus. Prepare the agarose gel just before the lab and maintain it in its liquid state by placing it in a 55–65°C water bath. Cover the top of the agarose container (flask) to minimize evaporation.

For eight-station kit: Add 5 grams of agarose to 625 mL of 1x TAE buffer that you prepared. Melt the agarose using a microwave (2–10 minutes) OR a hot plate with magnetic stir bar OR a boiling water bath. In all cases, heat the agarose until no particulate matter can be seen in the solution or stuck to the bottom of the flask. Be careful to prevent boiling over and/or scalding.

For four-station kit: Add 2.4 grams of agarose to 300 mL of 1x TAE buffer. Follow the instructions described above.

When the agarose cools (but before it solidifies), it is ready to be poured in the gel casting trays. Pour enough agarose into each tray until the volume is about halfway up each comb (approximately 50 mL per tray).

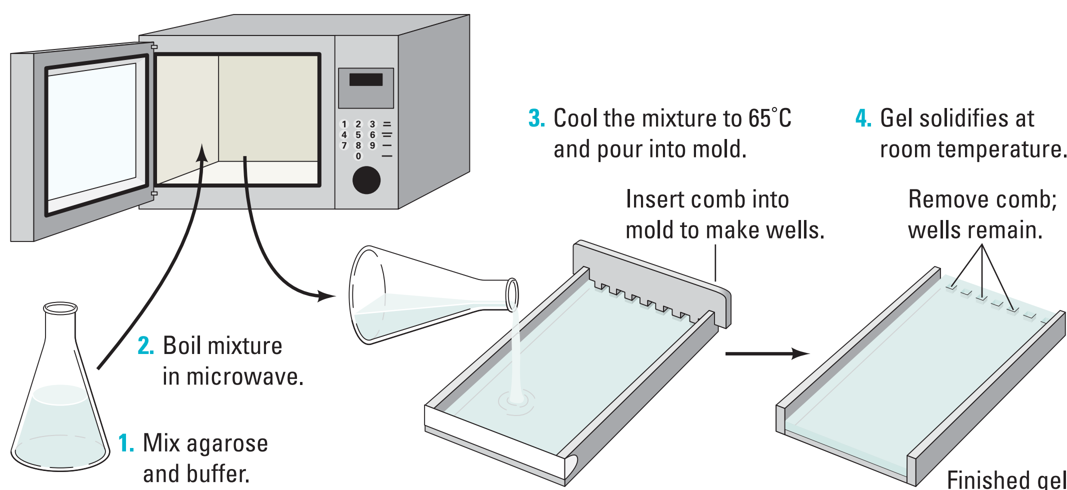


Figure 1. Preparing and Casting an Agarose Gel

Adding Methylene Blue Stain to Agarose

Adding gel and buffer stain to the gel and buffer allows observers to faintly see many of the bands (DNA fragments) in the gel while the electrophoresis is running, and this is instructive for students. It also helps you to monitor the progress of the electrophoresis. You will still have to use the final stain to see all the bands more clearly.

The concentration of the stain added to the agarose/buffer is dependent on the voltage used for electrophoresis. The stain may be added to the entire volume of the agarose. Just add the drops of stain to the agarose, swirl to mix, and pour the gel immediately. Gels can be prepared one day ahead of the lab day, if necessary. Gels stained longer than one day tend to fade and lose their ability to stain DNA fragments during electrophoresis. Refer to Table 1 for addition of stain to either 300 mL or 600 mL of agarose. Stain can be added using the dropper bottle. If a calibrated pipette is available, the lid of the dropper bottle can be removed for quicker addition of larger volumes.

CAUTION: Although methylene blue stain is not toxic, we recommend that you and your students wear gloves to prevent staining of the skin.

Table 1. Adding Methylene Blue Stain to Agarose

Voltage	Agarose Volume	Stain Volume
≤50 volts	300 mL	400 μL (10 drops)
	625 mL	760 μL (19 drops)
≥50 volts	300 mL	480 μL (12 drops)
	625 mL	1000 μL (25 drops)

Adding Methylene Blue Stain to Buffer

If you add stain to the gel, also add stain to the buffer. Use Table 2 to determine how much stain to add to either 500 mL or 2.6 L of 1x TAE electrophoresis buffer. However, if you plan to reuse the buffer, do not add stain to the buffer.

Table 2. Adding Methylene Blue Stain to Buffer

Voltage	Buffer Volume	Stain Volume
≤50 volts	500 mL	500 μL (12 drops)
	2.6 L	2.6 mL (65 drops)
≥50 volts	500 mL	960 μL (24 drops)
	2.6 L	5 mL (125 drops)

Staining with Final Stain

Methylene blue stain is added to the gel and buffer in order to better visualize the bands (fragments) of DNA as they migrate through the agarose gel during electrophoresis. However, you will still have to do a final stain of the gel.

1. Use the final stain "as is." Do *not* dilute it. Following electrophoresis, place the gel in the staining tray, and cover it with just enough stain to submerge the gel.

2. Use the methylene blue stain "as is." If you allow the gel to stain for more than an hour, the gel will be difficult to destain. It is helpful to set a timer.
3. Pour the stain back into the bottle for reuse; stain can be used six to eight times.
4. Destain the gels by covering them with distilled or deionized water. Do not use tap water because the chlorine in tap water will cause the DNA bands to fade. Change the water two or three times over the course of 30–40 minutes. Bands that are not immediately present will become more visible with time. Maximum visibility is reached after five or more hours of destaining. Gels may be left overnight in a small volume (just enough to cover the gels) of distilled or deionized water. Gels left overnight in a large volume of water may destain too much.

Timing and Length of Lab

Day Before the Lab

It is recommended that you prepare the TAE buffer and 0.8% agarose the day before the lab. This will take approximately 15 minutes. You will also need to spend about 30 minutes setting up student work stations and gathering electrophoresis equipment, preparing DNA "evidence" samples, test tube racks, etc. If you prefer to cast agarose gels for students, it will take approximately 20 minutes to pour gels into casting trays. The TAE buffer is stable and can be prepared ahead of time and stored.

Lab Day 1

Students will spend approximately 45 minutes reading the scenario and background material, working through several prelab activities, and familiarizing themselves with the procedure for gel electrophoresis. However, Getting Started can be assigned for homework. You can also have students view any of several online videos about DNA analysis using restriction enzymes and gel electrophoresis, including one on Carolina Biological Supply Company's website, for homework. Look for the Biotechnology link on the "Teacher Resources: Carolina Videos" Web page (***http://www.carolina.com***), or direct students to YouTube for educational videos and/or animations about preparing, pouring, loading, and running a gel.

Allow approximately 20 minutes for students to practice pipetting and gel loading (optional) and loading DNA samples in the gels. (See the Potential Challenges section of this investigation.)

It will take anywhere from 45 minutes to 2 hours to run the gels for the independent investigation, depending on the recommended voltage for the particular electrophoresis apparatus. For example, if you are using a standard-sized Carolina gel box (which is approximately eight inches long), run the gels at 135 volts. Using Bio-Rad gel boxes can take up to several hours at 50 volts. If the gels are run at a voltage that is too high, there will likely be less separation of the DNA fragments. Since students are likely to leave the lab to attend another class(es), you should plan on supervising the progress of the electrophoresis, stain the gels (15–30 minutes), and then destain them (45 minutes to overnight). Another suggestion is to have students come back to the lab at the end of school to stain and destain their gels.

Lab Day 2

Allow approximately 45 to 60 minutes for students to analyze, process, and discuss their results. It is recommended that you ask students to write conclusions to the scenario to reveal "whodunit" based on motive, means, opportunity, and, of course, DNA evidence. This project can be assigned for homework, but students will enjoy sharing their stories.

Safety and Housekeeping

Instruct students that they should never handle gels with their bare hands. An electrophoresis apparatus can be dangerous because it is filled with a highly conductive salt solution and uses DC current at a voltage strong enough to cause a small shock. Always turn the power supply switch "OFF" and wait 10 seconds before making any connection. Connect *both* supply leads to the power supply (black to black and red to red, just like when you jump-start a car battery) *before* turning on the power supply. After use, turn off the power supply, then disconnect *both* leads from the power supply. *Remember, power supply on last … and off first.*

ALIGNMENT TO THE AP BIOLOGY CURRICULUM FRAMEWORK

This investigation can be conducted during the study of concepts pertaining to the storage, retrieval, and transmission of genetic information (big idea 3), with connections to evolution (e.g., gene sequencing and measuring genetic variation, which is key to natural selection and evolution). As always, it is important to make connections between big ideas and enduring understandings, regardless of where in the curriculum the lab is taught. The concepts align with the enduring understandings and learning objectives from the AP Biology Curriculum Framework, as indicated below.

Enduring Understandings

- 1C3: Populations of organisms continue to evolve.
- 3A1: DNA, and in some cases RNA, is the primary source of heritable information.
- 3A3: The chromosomal basis of inheritance provides an understanding of the pattern of passage (transmission) of genes from parent to offspring.

Learning Objectives

- The student can justify the claim that humans can manipulate heritable information by identifying *at least two* commonly used technologies (3A1 & SP 6.4).
- The student is able to pose questions about ethical, social, or medical issues surrounding human genetic disorders [an application of genetic engineering] (3A3 & SP 3.1).

ARE STUDENTS READY TO COMPLETE A SUCCESSFUL INQUIRY-BASED, STUDENT-DIRECTED INVESTIGATION?

This investigation reinforces the following skills.

- Using pipettes (plastic bulb-type or other volumetric measuring devices) and other common lab equipment
- Graphing and applying other quantitative skills

Skills Development

Students will develop the following skills:

- Performing gel electrophoresis, a basic technique for separating DNA fragments by size
- Using more sophisticated mathematical practices, such as plotting data using the log scale

Potential Challenges

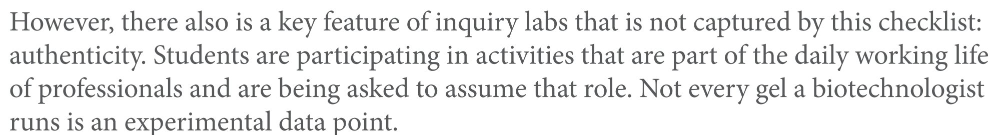
A major challenge for teachers is determining what electrophoresis conditions (i.e., voltage and time) provide the best results for the particular type of equipment. Please make sure to check the manufacturer's recommendations.

The biggest challenge for students is loading the small DNA samples into the wells. To save time, money, and resources, students should practice using either the plastic needle-nose pipettes provided or automatic pipettes before loading samples. Students will likely be a little nervous loading the samples, so you should show them how to use both hands to steady the pipette tip over the well. You should also caution students to not "punch through" the gel at the bottom of the well.

- Practice gels: The day before the lab, make one to two gels in a spare gel tray or rectangular plastic box using plain water and old agarose, old agar, or even unflavored gelatin (use three times the normal amount of gelatin called for on the package for a given volume of water). Put the comb in as usual, allow the gel to solidify, and then pull the comb out. Cover the gel with water and store refrigerated. These practice gels keep for a week and can be loaded and reloaded repeatedly by students; just use a plastic pipette and blow the practice samples out of the wells.
- Practice samples: Mix 10 drops of glycerol or corn syrup with 50 drops of water and one drop of blue food coloring. The final concentration of water may need to be more or less, depending on the density of the other liquids, so try pipetting the samples with your instruments.

Students should carefully read through the procedures *before* conducting the investigation, especially if this is the first time they have run an electrophoresis gel.

Despite the claim that they carefully loaded their samples into the wells of the gel, occasionally students will fail to get visible bands. If this happens, remind them that not all experiments work well all the time. Students must collect data from their gels, decide how to interpret and present their data, and make supportable conclusions.



However, there also is a key feature of inquiry labs that is not captured by this checklist: authenticity. Students are participating in activities that are part of the daily working life of professionals and are being asked to assume that role. Not every gel a biotechnologist runs is an experimental data point.

THE INVESTIGATIONS

Getting Started: Prelab Assessment

The Getting Started section of the student version of this investigation provides students with three activities to review or introduce key concepts and principles relating to restriction enzymes, DNA mapping using restriction enzymes, and analysis of DNA using gel electrophoresis. These activities encourage students to ask questions as they take a deeper dive into the concepts. You may assign these activities for homework or ask students to do them as they work through the investigation. Student lab groups can share questions they raise — and answers to questions they investigate — with other groups. You can follow up by asking more probing questions.

Procedures

To determine whose blood was on the classroom floor, students must first familiarize themselves with the techniques involved in creating genetic profiles using gel electrophoresis and calculating the sizes of restriction length polymorphisms (RFLPs). Students then analyze profiles resulting from an "ideal" or mock gel *before* running an actual gel.

Designing and Conducting Independent Investigations

Students use their newly learned technical skills to design and conduct a procedure *based on DNA evidence* to determine whose blood is spattered on the classroom floor in the crime scene scenario. Creating DNA profiles, students narrow the list of suspects in the disappearance of Ms. Mason and ultimately determine "whodunit" based on motive, means, opportunity, and, of course, science.

There is no single correct answer to "whodunit" in the crime scene scenario provided. By preparing different samples of DNA "evidence," you can take the scenario in multiple directions with multiple suspects. For example, Bobby could have cut himself on a flask he accidentally broke while tossing a basketball around the classroom. (This could present an opportunity to review lab safety.) Or Mr. Gladson, Ms. Mason's rival for promotion to chair of the department, might have taken his disappointment a bit too far. Maybe Ms. Mason staged the scene as a hands-on quiz on processing a crime scene for her forensic science course.

The scenario is fictitious, and there is no reference to the teacher, Ms. Mason, having been murdered; she has simply disappeared. (Note: A reliable source reported that Ms. Mason recently was seen relaxing poolside at a resort following an exhausting year teaching AP Biology.)

Where Can Students Go from Here?

Another suggestion, especially if conducted in concert with a civics class, would be to stage a mock trial at which the data are presented. For example, the defense would be required to argue for the unreliability of the data, while the prosecution would have to present it clearly. Or one of the suspects in the scenario could be brought to trial for his or her role in the disappearance of Ms. Mason.

Summative Assessment

The following are guidelines to assess students' understanding of the concepts presented in the investigation, but you are encouraged to develop your own methods of postlab assessment. Some of the tasks can be assigned for homework following the completion of the investigation.

1. Have students record all their data, results, and conclusions in a lab notebook, formal paper, or mini-posters. Based on the students' product, do you think students have met the learning objectives of the investigation?
2. As you visited the different lab groups, were they able to work through the various activities interspersed throughout the investigation without difficulty? What additional questions did students raise? Did they have ideas for how they could explore answers to their questions?
3. Were students able to plot data on semi-log paper, construct a standard curve, and then determine the approximately lengths (in bp) of unknown fragments using the standard curve?
4. What technical challenges did students have using the equipment required for the investigation? Have them list their challenge areas and discuss solutions.

SUPPLEMENTAL RESOURCES

Prelab Activities

The Getting Started section of the investigation contains myriad inquiry-based questions for preparing students, and encourages them to ask their own questions and explore answers. Interspersed within the investigation are activities designed to keep students on track and to provide opportunities for them to take a deeper dive into the concepts. You may assign these activities for homework or ask that students do them as they work through each part of the investigation.

http://biology.arizona.edu

The University of Arizona Biology Project is an online interactive resource for learning biology, with an extensive molecular biology/biotechnology module.

Curriculum Module (Professional Development), AP Biology: *From Gene to Protein—A Historical Perspective*, College Board, 2010.
This set of instructional strategies developed by AP Biology teachers takes students on an inquiry-based journey as they explore key discoveries that allowed scientists to identify DNA as *the* molecule of heredity and how it is able to store, retrieve, and transmit information necessary for living systems. Drawing their own conclusions, students explore the contributions of notable scientists, including Frederick Griffith, Hershey and Chase, Watson and Crick, and Meselson and Stahl. The instructional activities are examples of how teachers can engage students by accommodating their different learning styles, knowledge bases, and abilities and, at the same time, provide depth of content and skills.

http://dnalc.org Dolan DNA Learning Center, Cold Spring Harbor.
This resource provides myriad interactive activities for students to prepare students for conducting investigations using biotechnology practices, including DNA Subway and iPlant Collaborative.

http://phschool.com/science/biology_place
Developed by Pearson Education, this interactive and informative resource allows students to visualize and apply their understanding of biological concepts. Designed for AP Biology students, LabBench connects laboratory procedures to key concepts.

Procedural Resources

Molecular Biology and Biotechnology: A Guide for Teachers, Carolina Biological item RN-212240

http://www.neb.com
New England Biolabs has an extensive selection of DNA markers cut with several restriction enzymes, including *Hin*dIII, *Hae*III, and *Bst*EII, as well as standardized 1000-bp and 100-bp standards. NEB also sells ladder DNA made from plasmids (pBR322) and the Phi-Chi X174 virus, giving teachers options for "suspect" DNA samples in the crime scene scenario. The NEB catalog (both in print and online) is a treasure trove of information, including images of the precut DNAs. Their 1kb and 100bp ladders are particularly helpful for demonstrating the log relationship between mobility and size.

Restriction Enzyme Cleavage of DNA Kit, Carolina Biological (catalog number 21149), 2010. **http://www.carolina.com**

The two resources above provide detailed background information and dry labs for teaching about restriction enzymes and gel electrophoresis.

Resources for Extensions of Investigation

Biotechnology Explorer™ Forensic DNA Fingerprinting Kit, Catalog #166-0007EDU. ***http://explorer.bio-rad.com***

This resource provides an extensive curriculum of activities for students based on DNA fingerprinting. Teachers can "pick and choose" appropriate explorations depending on student interest and ability, including applications of PCR, VNTRs, and STRs.

Biotechnology Explorer™ Cloning Sequencing Explorer Series, Catalog #166-5000EDU. ***http://explorer.bio-rad.com***

This resource provides an extensive research project composed of eight lab modules which can be used separately or in a series for an entire six-to-eight-week project. Due to the modular natures of the series, some components are used in conjunction with other modules for a continuous workflow. The series modules are Nucleic Acid Extraction, *GADPH* PCR, Electrophoresis, PCR Kleen™ Spin Purification, Ligation and Transformation, Microbial Culturing, Aurum™ Plasmid Mini Purification, and Sequencing and Bioinformatics. The module is geared toward small class sizes of advanced students. The estimated price of the module is $1,400.

Brown, Betty, et al., *Get a Clue*, Destiny, University of North Carolina at Chapel Hill, 2006. ***https://www.bio-rad.com/cmc_upload/Products/-41683/Get_A_Clue_DESTINY.pdf***

This resource provides an extensive curriculum of activities for students based on DNA fingerprinting. Teachers can "pick and choose" appropriate explorations depending on student interest and ability, including dry labs to introduce PCR and VNTR/STR analysis.

Gattaca, Columbia Pictures, 1997, PG-13.

The movie available on DVD transports us into a future society (Gattaca) defined by genetic discrimination when a genetically inferior man assumes the identity of a superior one in order to pursue his lifelong dream of space travel. The issues that are raised, including questions about the social and ethical implications of DNA analysis, provide fodder for discussion and debate.

http://innocenceproject.org

This resource provides information on The Innocence Project (IP), an international litigation and public policy organization dedicated to exonerating wrongfully convicted individuals through DNA testing.

Johnson, A. Daniel, *40 Exercises for the College Biology Lab*, NSTA Press, Arlington, VA, 2009.

This information provides great insight into developing student-directed, inquiry-based laboratory investigations for advanced students, while also providing strategies on how teachers can adapt their more teacher-directed labs to provide opportunities for independent exploration. Unit 3 in the manual, "DNA Isolation and Analysis," provides exercises for more advanced students to use bioinformatics programs to study and manipulate DNA sequences.

This page is intentionally left blank.

INVESTIGATION 9

BIOTECHNOLOGY: RESTRICTION ENZYME ANALYSIS OF DNA*

How can we use genetic information to identify and profile individuals?

THE SCENARIO

"OMG! Is that blood?" Laurel nearly broke Marcus's arm as she tried to push past him into the classroom.

Marcus grabbed the sleeve of her cardigan and yanked her back. "Don't! Can't you see the glass?" Laurel tried knocking his hand free, but the 6'4" varsity basketball captain held tight. He made her settle for looking from under his armpit.

Not that what she saw would make any sense. Their AP Biology lab looked like a riot scene. Four chairs and a potted plant were overturned in the center of the room, and broken pieces of glass were scattered across the floor along with several wet red drops. Plink ... plink ... plink. *Marcus's eyes were drawn to the teacher's desk where droplets of brownish liquid fell from a paper cup and collected in a puddle on the linoleum.*

"What happened?" Laurel asked. "Did somebody get hurt?" Laurel and her classmates had gathered in front of the door and strained to see inside Room 102.

Marcus inspected the scene and raised his right arm above his head, his fingers spread apart as if taking a shot from the free throw line. "Stay back!"

"Where's Ms. Mason?" Laurel said. "She told me I could meet her before class to review for the quiz."

"Okay, folks, keep it down." Mr. Gladson, the teacher in the classroom next door, came into the hall. His white lab coat was streaked with several rust-colored stains. The pungent odor of formaldehyde permeated the corridor. "In case you haven't noticed, the bell has rung." He wiped his nose with a tissue and then tossed it into a nearby trash can. A girl's fake shriek from inside the anatomy lab rose above the buzz of Marcus's classmates.

"What's going on?" Bobby's high-pitched whine was unmistakable — and so was the scent of his bubble gum.

"I think something might've happened to Ms. Mason," Marcus said. He dug around in his backpack and pulled out a magnifying glass. "We've got a crime scene to process."

---

* Transitioned from the *AP Biology Lab Manual* (2001)

"Go figure," Laurel said. "Sherlock Holmes in a varsity jacket."

For the next hour, Marcus and Laurel searched the classroom and discovered several pieces of "evidence" that Marcus described in his biology notebook:

- Ten small drops on floor confirmed by Kastle-Meyer test to be blood
- Shard of glass from a broken 500-mL Erlenmeyer flask, edge smeared with a reddish stain
- Paper cup with lipstick stains, presumed to be Ms. Mason's, found on her desk
- Wad of bubble gum stuck underneath overturned chair
- Mr. Gladson's discarded tissue recovered from trash can in hall outside Room 102
- Bobby's test on photosynthesis with large "F" scrawled in red ink on first page
- Copy of email from Mr. Gladson to Ms. Mason asking her to give up position as department chair

Marcus's new game was afoot!

BACKGROUND

Applications of DNA profiling extend beyond what we see on television crime shows. Are you sure that the hamburger you recently ate at the local fast-food restaurant was actually made from pure beef? DNA typing has revealed that often "hamburger" meat is a mixture of pork and other nonbeef meats, and some fast-food chains admit to adding soybeans to their "meat" products as protein fillers. In addition to confirming what you ate for lunch, DNA technology can be used to determine paternity, diagnose an inherited illness, and solve historical mysteries, such as the identity of the formerly anonymous individual buried at the Tomb of the Unknown Soldier in Washington, D.C.

DNA testing also makes it possible to profile ourselves genetically — which raises questions, including *Who owns your DNA and the information it carries?* This is not just a hypothetical question. The fate of dozens of companies, hundreds of patents, and billions of dollars' worth of research and development money depend on the answer. Biotechnology makes it possible for humans to engineer heritable changes in DNA, and this investigation provides an opportunity for you to explore the ethical, social, and medical issues surrounding the manipulation of genetic information.

Learning Objectives

In this investigation, you will learn how to use restriction enzymes and gel electrophoresis to create genetic profiles. You will use these profiles to help Marcus and Laurel narrow the list of suspects in the disappearance of Ms. Mason.

General Safety Precautions

Never handle gels with your bare hands. An electrophoresis apparatus can be dangerous because it is filled with a highly conductive salt solution and uses DC current at a voltage strong enough to cause a small shock. Always turn the power supply switch

"OFF" and wait 10 seconds before making any connection. Connect BOTH supply leads to the power supply (black to black and red to red, just like when you jump-start a car battery) BEFORE turning on the power supply. Your teacher will tell you for how long and at how many volts (usually 50 volts) you should run your gel. After use, turn off the power supply, and then disconnect BOTH leads from the power supply. *Remember, power supply on last … and off first.*

THE INVESTIGATIONS

Getting Started

Activity I: Restriction Enzymes

The DNA samples collected from the crime scene have been digested with restriction enzymes to generate smaller pieces of DNA, which will then be used to create DNA profiles of suspects.

Restriction enzymes are essential tools for analyzing DNA structure, and more than 200 enzymes are now available commercially. Each restriction enzyme is named for the bacterium in which it was first identified; for example, *Eco*RI was the first enzyme purified from *Escherichia coli,* and *Hin*dIII was the third enzyme isolated from *Haemophilus influenzae*. Scientists have hypothesized that bacteria use these enzymes during DNA repair and as a defense against their infection by bacteriophages. Molecular biologists use restriction enzymes to manipulate and analyze DNA sequences (Johnson 2009).

How do restriction enzymes work? These enzymes digest DNA by cutting the molecule at specific locations called restriction sites. Many restriction enzymes recognize a 4- to 10-nucleotide base pair (bp) palindrome, a sequence of DNA nucleotides that reads the same from either direction. Some restriction enzymes cut (or "cleave") DNA strands exactly in the center of the restriction site (or "cleavage site"), creating blunt ends, whereas others cut the backbone in two places, so that the pieces have single-stranded overhanging or "sticky" ends of unpaired nucleotides.

You have a piece of DNA with the following template strand:

5'-AAAGTCGCTGGAATTCACTGCATCGAATTCCCGGGGCTATATATGGAATTCGA-3'

1. What is the sequence of the complementary DNA strand? Draw it directly below the strand.
2. Assume you cut this fragment with the restriction enzyme *Eco*RI. The restriction site for *Eco*RI is 5'-GAATTC-3', and the enzyme makes a *staggered* ("sticky end") cut between G and A on both strands of the DNA molecule. Based on this information, draw an illustration showing how the DNA fragment is cut by *Eco*RI and the resulting products.

Two pieces of DNA that are cut with the same restriction enzyme, creating either sticky ends or blunt ends, can be "pasted" together using DNA ligase by reconnecting bonds, *even if the segments originated from different organisms.* An example of combining two "sticky end" sequences from different sources is shown in Figure 1. The ability of enzymes to "cut and paste" DNA fragments from different sources to make recombinant DNA molecules is the basis of biotechnology.

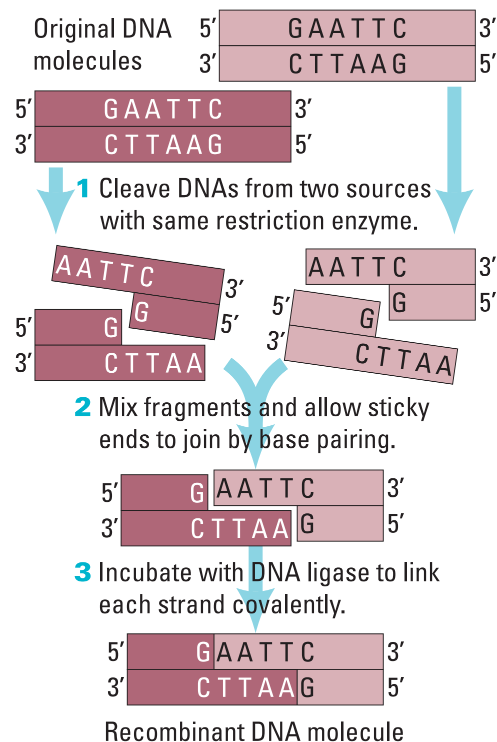


Figure 1. Recombinant DNA Using Restriction Enzymes

Activity II: DNA Mapping Using Restriction Enzymes

One application of restriction enzymes is restriction mapping. Restriction mapping is the process of cutting DNA at specific sequences with restriction enzymes, separating the fragments from each other by a process called gel electrophoresis (without pasting any fragments together), and then estimating the size of those fragments. The size and number of DNA fragments provide information about the structure of the original pieces of DNA from which they were cut.

Restriction mapping enables scientists to create a genetic signature or DNA "fingerprint" that is unique to each organism. The unique fragments, called restriction fragment length polymorphisms (RFLPs), can, for instance, be used to confirm that a mutation is present in one fragment of DNA but not in another, to determine the size of an unknown DNA fragment that was inserted into a plasmid, to compare the genomes of different species and determine evolutionary relationships, and to compare DNA

samples from different individuals within a population. This latter application is widely used in crime scene investigations.

Consider your classmates. More than 99% of your DNA is the same as their DNA. The small difference is attributed to differences in your genetic makeup, with each person having a genetic profile or "fingerprint" as unique as the ridges, arches, loops, and grooves at the ends of his or her fingers.

- Based on this information, can you make a prediction about the products of DNA from different sources cut with the same restriction enzymes? Will the RFLP patterns produced by gel electrophoresis produced by DNA mapping be the same or different if you use just one restriction enzyme? Do you have to use many restriction enzymes to find differences between individuals? Justify your prediction.
- Can you make a prediction about the RFLP patterns of identical twins cut with the same restriction enzymes? How about the RFLP patterns of fraternal twins or triplets?

Now that you understand the basic idea of genetic mapping by using restriction enzymes, let's explore how DNA fragments can be used to make a genetic profile.

Activity III: Basic Principles of Gel Electrophoresis

Creating DNA profiles depends on gel electrophoresis. Gel electrophoresis separates charged molecules, including nucleic acids and amino acids, by how fast they migrate through a porous gel under the influence of an electrical current. Your teacher will likely prepare the gel ahead of time by dissolving agarose powder (a gelatinlike substance purified from seaweed) in a current-carrying buffer. The gel solidifies around a comb placed at one end, forming wells into which you can load DNA fragments. When an electrical current is passed through the gel, the RFLPs (fragments) migrate from one pole to the other. Gel electrophoresis can separate DNA fragments from about 200 to 50,000 base pairs (bp).

- Why do DNA fragments migrate through the gel from the *negatively* charged pole to the *positively* charged pole?

The general process of gel electrophoresis is illustrated in Figure 2.

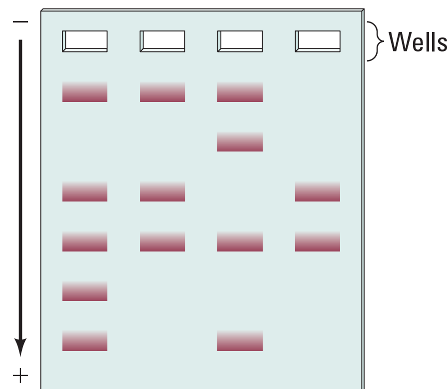


Figure 2. General Process of Gel Electrophoresis

Procedures

Learning to Use Gel Electrophoresis

To determine whose blood was on the classroom floor crime scene, you will need to be familiar with the techniques involved in creating genetic profiles using gel electrophoresis. The steps in the general procedure are described below. After you familiarize yourself with the procedure, you will analyze DNA profiles resulting from an "ideal" or mock gel before using what you have learned to conduct an independent investigation. In Designing and Conducting Your Investigation, you will use these skills to narrow the list of suspects in the disappearance of Ms. Mason based on DNA evidence collected at the crime scene.

Materials

Your Workstation

- 20 µL vials of DNA fragments prepared using restriction enzymes
- Rack for holding samples
- 3 plastic bulb transfer pipettes (or similar devices)
- Permanent marker
- Gel electrophoresis chamber
- Power supply
- Staining tray
- Semi-log graph paper
- Ruler

Common Workstation

- 0.8% agarose solution (or gel, if prepared by teacher)
- 1 X TAE (tris-acetate-EDTA) buffer
- Methylene blue stain

Record data and any answers to questions in your lab notebook.

Casting the Agarose Gel

Before proceeding, your teacher will direct you to short online videos that show how to prepare an agarose gel, load DNA samples into the wells in the gel, and run an electrophoresis.

Step 1 Seal the ends of the gel-casting tray with tape, dams, or any other method appropriate for the gel box that you are using. Insert the well-forming comb. Place the gel-casting tray out of the way on the lab bench so that the agarose poured in the next step can set undisturbed. (Your teacher might cast the gel for you ahead of time.)

Step 2 Carefully pour the liquid gel into the casting tray to a depth of 5–6 mm. The gel should cover only about one-half the height of the comb teeth (Figure 3). While the gel is still liquid, use the tip of a pipette to remove any bubbles.

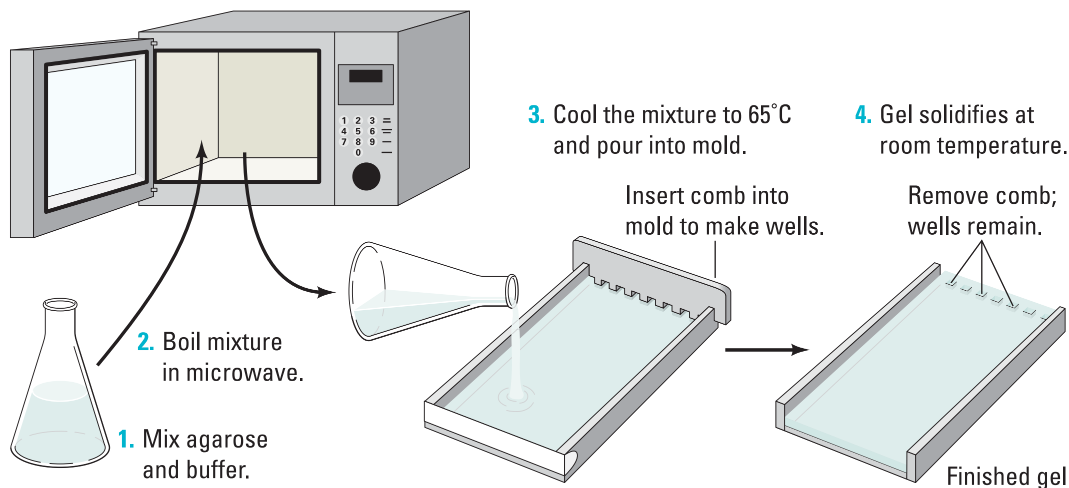


Figure 3. Casting an Agarose Gel

Step 3 The gel will become cloudy as it solidifies (15–20 minutes). Do not disturb or touch the gel while it is solidifying!

Step 4 When the agarose has set, carefully remove the ends of the casting tray and place the tray in the electrophoresis gel box so that the comb is at the negative (black) end.

- Why do you place the wells at the negative end of the gel box?
- What is the chemical nature of DNA? Will the DNA fragments migrate toward the positive end of the gel box or toward the negative end?

Step 5 Fill the box with 1x TAE buffer, to a level that just covers the entire surface of the gel.

Step 6 Gently remove the comb, taking care not to rip the wells. Make sure that the sample wells left by the comb are completely submerged in the buffer.

Step 7 The gel is now ready to be loaded with your DNA samples. (If your teacher says that you will load the gel on another lab day, close the electrophoresis box to prevent drying of the gel.)

Loading the Gel

Before loading your gel with samples of DNA, you should practice using the pipette or other loading device. One easy way to do this is to slowly aspire a sample of buffer and expel it into a "pretend well" on a paper towel ("pretend gel"). Your teacher might suggest another method for practicing how to load gels. Keep practicing until you feel comfortable loading and expelling a sample.

Make sure you record the order in which you load the samples. Be sure to use a fresh loading device (either plastic micropipette or other type of pipette) for each sample. Be sure you know how to use the pipette properly. When in doubt, ask your teacher. Take care not to puncture the bottom of the well with the pipette tip when you load your samples.

Step 1 Load 15–20 μL of each sample of DNA into a separate well in the gel, as shown in Figure 4.

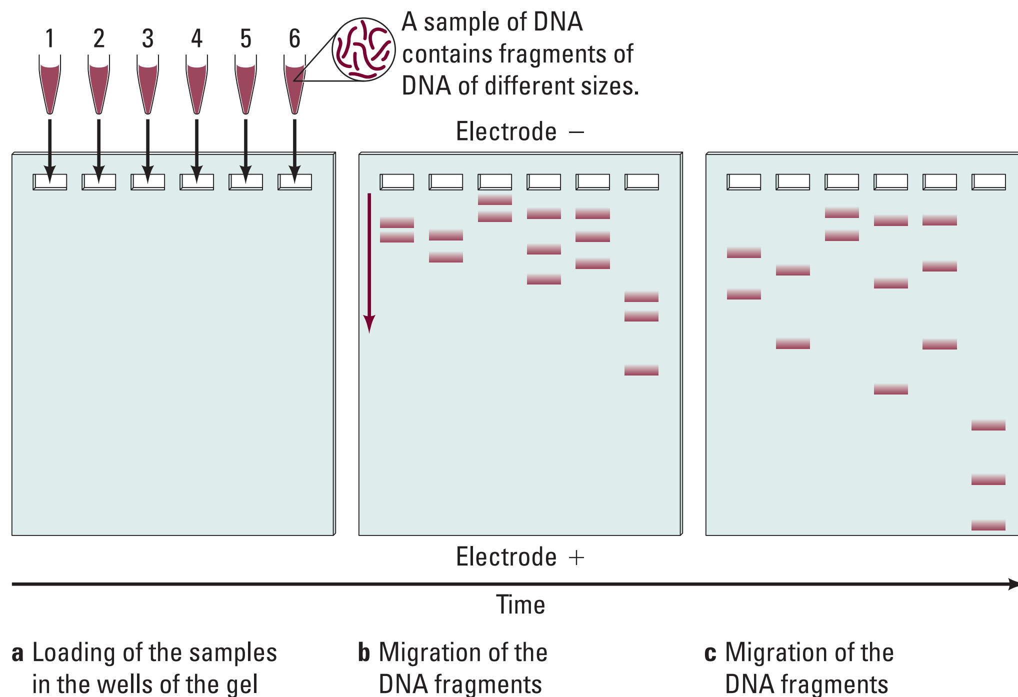


Figure 4. Loading an Agarose Gel and Migrating DNA Fragments Through Time

Step 2 Slowly draw up the contents of the first sample tube into the pipette.

Step 3 Using two hands, steady the pipette over the well you are going to load.

Step 4 Expel any air in the end of the pipette *before* loading the DNA sample.

Step 5 Dip the pipette tip through the surface of the buffer, position it just inside the well, and slowly expel the mixture. Sucrose in the loading dye weighs down the sample, causing it to sink to the bottom of the well. *Be careful not to puncture the bottom of the well with the pipette tip or reaspirate your sample up into the pipette.*

Step 6 Draw the pipette tip out of the buffer.

Step 7 Using a clean loading device for each sample, load the remaining samples into their wells.

Electrophoresis

Step 1 Close the top of the electrophoresis chamber and then connect the electrical leads to an appropriate power supply, positive (+) electrode to positive (+) electrode (red to red) and negative (-) electrode to negative (-) electrode (black to black). Make sure both electrodes are connected to the same channel of the power supply, just as you would connect leads to jump-start a car battery — red to red and black to black.

CAUTION: Be sure to keep the power OFF until you connect all leads!

Step 2 Turn on the power supply and set the voltage as directed by your teacher. (It is recommended that you "run the gel" at 50 volts for approximately 2 hours. If you run the gel at a higher voltage for less time, the fragments migrate too quickly through the gel with less separation. Again, ask your teacher for assistance if needed.)

Step 3 Shortly after the current is applied, you should see loading dye moving through the gel toward the positive pole of the electrophoresis apparatus. (**Note:** The purplish-blue band in the loading dye migrates through the gel at the same rate as a DNA fragment approximately 300 base pairs long.)

Step 4 Allow the DNA to electrophorese until the loading dye band is about 1 cm from the end of the gel. Your teacher may monitor the progress of the electrophoresis in your absence if you have to attend another class.

Step 5 Turn off the power supply, disconnect the leads from the power supply, and remove the lid of the electrophoresis chamber.

Step 6 Carefully remove the casting tray and slide the gel into a staining tray labeled with the name of your group.

- Measure in centimeters the distance that the purplish-blue loading dye has migrated into the gel. Measure from the front edge of the well to the front edge of the dye band (also called the dye front).
- Be sure to record your data (in centimeters).

Step 7 Take your gel to your teacher for further staining instructions. Again, your teacher might monitor the staining procedure.

Analyzing Results

Calculating the Sizes of Restriction Fragment Length Polymorphisms

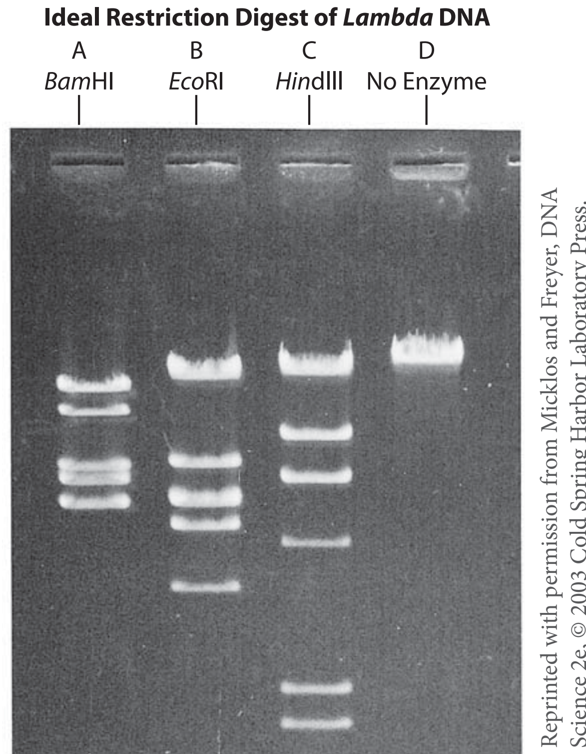
Mathematical formulas have been developed for describing the relationship between the molecular weight of a DNA fragment and its mobility (i.e., how far it migrates in the gel). In general, DNA fragments, like the ones in your evidence samples, migrate at rates inversely proportional to the $\log_{10}$ of their molecular weights. **For simplicity's sake, base pair length (bp) is substituted for molecular weight when determining the size of DNA fragments.** Thus, the size in base pair length of a DNA fragment can be calculated using the distance the fragment travels through the gel. To calculate the base pair length, a DNA standard, composed of DNA fragments of *known* base pair length, is run on the same gel as the unknown fragments and is then used to create a standard curve. The standard curve, in this case a straight line, is created by graphing the distance each fragment traveled through the gel versus the $\log_{10}$ of its base pair length.

Creating the Standard Curve

As explained above, base pair (bp) length is substituted for molecular weight. Note that in plotting the standard curve, calculating the $\log_{10}$ of the base pair length of each fragment is unnecessary because the base pair size is plotted on the logarithmic axis of semi-log paper. Examine your stained gel on a light box or other surface that helps visualize the bands.

- What observations can you make?
- What quantitative measurements can you make?

1. Examine the "ideal" or mock gel shown in Figure 5 that includes DNA samples that have been cut with three restriction enzymes, *Bam*HI, *Eco*RI, and *Hin*dIII, to produce RFLPs (fragments). Sample D is DNA that has not been cut with enzyme(s). DNA cut with *Hin*dIII provides a set of fragments of known size and serves as a standard for comparison.



Reprinted with permission from Micklos and Freyer, DNA Science 2e, © 2003 Cold Spring Harbor Laboratory Press.

Figure 5. Ideal Gel

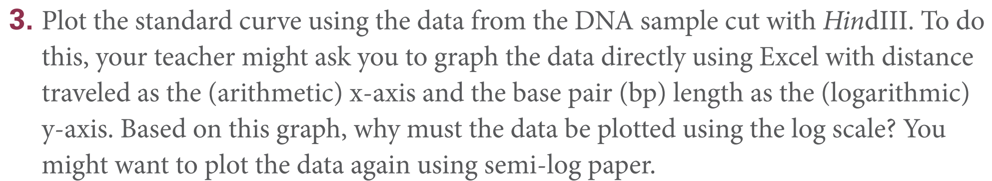
2. Using the ideal gel shown in Figure 5, measure the distance (in cm) that each fragment migrated *from* the origin (the well). (**Hint:** For consistency, measure from the front end of each well to the front edge of each band, i.e., the edge farthest from the well.). Enter the measured distances into Table 1. (See * and ** notes below the table for an explanation for why there are only six bands seen but more fragments.)

Table 1. DNA Fragment Migration Distance

*HIN*DIII		*BAM*HI		*ECO*RI	
Distance Traveled	BP Length	Distance Traveled	BP Length	Distance Traveled	BP Length
	*27,491				
	*23,130				
	9,416				
	6,557				
	4,361				
	2,322				
	2,027				
	**564				
	**125				

*For this "ideal" gel, assume that these two bands appear as a single band instead of resolving into separate bands.

** These bands do not appear on the ideal gel and likely will not be seen.



3. Plot the standard curve using the data from the DNA sample cut with *Hin*dIII. To do this, your teacher might ask you to graph the data directly using Excel with distance traveled as the (arithmetic) x-axis and the base pair (bp) length as the (logarithmic) y-axis. Based on this graph, why must the data be plotted using the log scale? You might want to plot the data again using semi-log paper.

   Connect the data point with a best-fit line. However, you should ignore the point plotted for the 27,491bp/23,130 doublet. When using 0.8% agarose gel, these fragments appear as one. Congratulations! Your best-fit line is the standard curve.

4. Now use the standard curve to calculate the approximate sizes of the *Eco*RI and *Bam*HI fragments. Using a ruler, how can you use the standard curve to calculate the sizes of unknown fragments?

Designing and Conducting Your Investigation

Now that you've learned about the techniques used to create DNA profiles or "fingerprints," it's time to apply the techniques as you investigate the disappearance of Ms. Mason. Your task is to design and conduct a procedure *based on DNA evidence* to determine whose blood is spattered on the classroom floor. The chief investigator (your teacher) will provide you with DNA evidence collected at the crime scene from the blood, Ms. Mason (saliva on her coffee cup), Mr. Gladson (tissue with which he wiped his nose), and Bobby (bubble gum). In addition, you will be given a sample of DNA cut with *Hin*dIII. Remember from your analysis of the "ideal" or mock gel that DNA cut with *Hin*dIII serves as a marker, providing a set of RFLPs of known sizes (standard).

Analyzing Results

Evaluate your crime scene samples to determine whose blood was on the classroom floor. Because this case likely will go to trial, visual analysis (qualitative data) of the DNA profiles is not sufficient to identify a perpetrator. Based on your results, write the conclusion to the scenario to reveal "whodunit" based on motive, means, opportunity, and DNA evidence.

Evaluating Results

1. What are some possible challenges you had in performing your investigation?

2. What are some possible sources of error in the electrophoresis procedure? How can you minimize any potential sources of error?

Thinking About Your Results

1. There are important social and ethical implications of DNA analysis. Already, DNA testing can reveal the presence of markers of certain genetic diseases, such as Huntington's. So, who should have access to your genetic profile? Health insurance companies? College admissions offices? Employers? What issues about confidentiality are raised by genetic testing? Who owns your DNA and its information?

2. Suppose a DNA test that predicted your chances of getting a disease, such as cancer, were available. You take the test for cancer, and the results say you have a two in three chance of getting cancer sometime in the next 20 years. Who should have access to this information? Your doctor? Health insurance companies? Employers? Would *you* want to know this information?

3. The Innocence Project (IP) is an international litigation and public policy organization dedicated to exonerating wrongfully convicted individuals through DNA testing. Three-quarters of DNA exoneration cases involve misidentification by witnesses. To date, nearly 300 people previously convicted of serious crimes in the U.S. have been exonerated by DNA testing. However, not everyone is in favor of the IP. One United States Supreme Court justice expressed concern that DNA testing poses risks to the criminal justice system, in which a person is judged by a jury of peers. What social and ethical issues are raised by using DNA evidence to re-examine old court decisions? What other arguments can you make (or find) against using DNA evidence for court cases?

4. With genetic engineering, biotechnicians can clip out beneficial genes from native plants in foreign countries and insert them into their crop plant relatives here in the United States, with great benefits to the latter — to prevent attack by insects, to increase productivity, or to allow the crops to be grown in colder climates. These benefits can be worth billions of dollars, but if the crops are grown in the United States, should countries where the native plants are located benefit from the bioengineering? Who owns the information in DNA? Who can profit from that information? Investigate this controversy on the Internet with examples drawn from different crops grown here in the U.S.

Where Can You Go from Here?

The following are suggestions for expanding your study of biotechnology.

1. Do you remember earlier when you read that more than 99% of your DNA is the same as another person's DNA, and that the 1% difference is attributed to small differences in genetic code? Conduct independent research on how these small differences can be detected by molecular biologists. Begin by investigating unique repeat DNA sequences called variable tandem repeats (VNTRs), short tandem repeats (STRs), and single nucleotide polymorphisms (SNPs). Prepare a mini-poster presentation for your classmates illustrating how these small differences can be used to individualize DNA from different organisms, including humans. Are the differences between you and other individuals in the genes themselves? If so, how do you account for the fact that everyone needs the same genes to produce your cell components and your organs, such as your liver and lungs?

2. Often scientists have only a small amount of DNA available for analysis. The polymerase chain reaction (PCR) is another key technique that molecular biologists use to amplify a specific sequence of DNA. Developed by Kary Mullis in 1983, PCR produces millions of copies of a DNA sequence in a few hours, with the original sequence serving as the template for replication. PCR has a variety of applications, including DNA cloning, determining DNA-based phylogeny, diagnosing hereditary diseases, and identifying genetic fingerprints. Ask your teacher if you can learn to perform PCR. PCR usually requires a relatively expensive piece of equipment, a DNA thermocycler; however, you can investigate less expensive methods of PCR.

3. Select an episode of one of your favorite TV crime investigation shows that focuses on DNA as evidence. Compare TV science with *real* science.