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 workstations. **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).

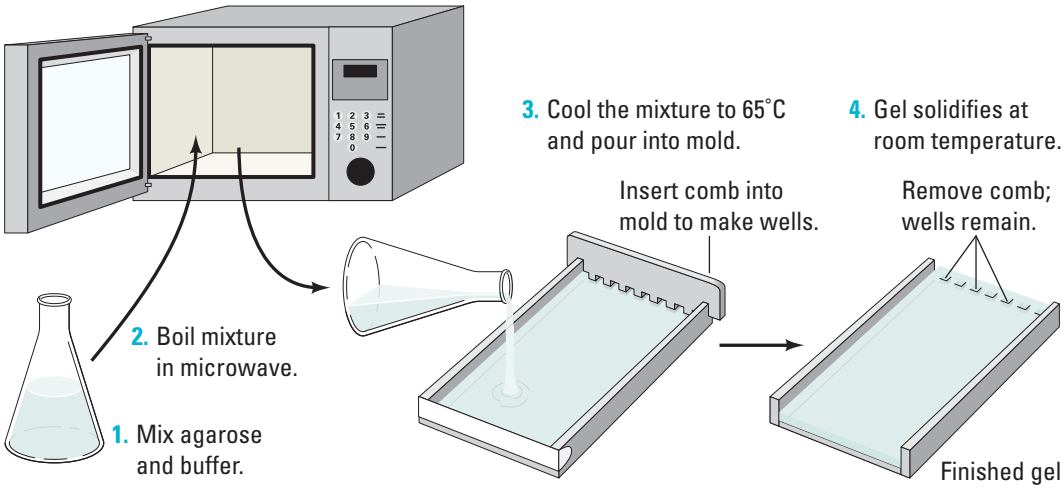

Figure 1. Preparing and Casting an Agarose Gel

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.

http://www.carolina.com/category/teacher%20resources/educational%20videos.do
This resource is one of several online videos about DNA analysis using restriction enzymes and gel electrophoresis. It is appropriate for students to view for prelab preparation.

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://www3.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.