Two Simple and Inexpensive Laboratory Exercises for Teaching Agarose Gel Electrophoresis and DNA Fingerprinting

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Summary. **One of the most frequently used tools in plant biotechnology, which includes genomics and proteomics, is gel electrophoresis. Our experience with middle and high school students as well as teachers and undergraduate students is that they have very little, if any, hands-on experience with this technique. These exercises were developed to demonstrate the principles of electrophoresis and DNA fingerprinting in middle and high school and university laboratories with minimal expense and equipment. The experiments have been tested by middle and high school students, as well as by teachers, and undergraduate and graduate students. The first exercise, electrophoresis of common food dyes, is primarily designed for secondary and undergraduate students, but can be used as an inexpensive means for introducing the main concepts of electrophoresis to anyone who has little or no experience, including graduate students. Popular brands of food dyes (red, blue, yellow, and green) purchased at local markets are mixed into a 60% glycerol/water solution and are separated on 1% agarose gels using 100 V for 35 min. Mixed colors are separated into primary colors (e.g., green into blue and yellow) and some apparently single dyes often have extra "surprise" components. A simple exercise illustrating forensic use of gel electrophoresis with dyes is also included. Over 100 students and teachers have completed this experiment successfully. The second laboratory exercise requires more extensive equipment and a more advanced set of skills; however, the exercise has been completed successfully by middle school-level through graduate-level students and by teachers. In this exercise, the internally transcribed spacer region of the ribosomal subunit for a fungus, plant, and insect are amplified and separated electrophoretically on agarose gels. A simple crime is solved using polymerase chain reaction (PCR) and DNA fingerprinting. The experiment protocol provides students with hands-on activities that include assembling master mixes for PCR, practice using pipettes, and performing the various steps involved in PCR amplification. Instructions for both exercises are formatted in easy-to-follow procedure boxes, and a downloadable presentation is available on the web. The cost of the expendables is about $1 per student, making these exercises relatively inexpensive to conduct, assuming that hardware and DNA are available.**

Biotechnology is a rapidly evolving field of science that combines cellular and molecular biology with applications in genetic engineering and recombinant DNA technology. The tools of biotechnology have come to play an increasingly important role in our everyday lives, from rapid detection and diagnosis of diseases in humans, plants, and animals to genetic manipulation for improvement of plants or animals or development of microorganisms for specific uses. Understanding key concepts in biotechnology will give students in middle and high school and college the information they need to critically evaluate and make crucial decisions in the future about societal issues related to human health, food safety and production, and preservation of the environment.

A concern among many is that a lack of skilled human resources is a major constraint to development of biotechnology industries. Therefore, it is important to introduce biotechnology concepts sufficiently early in the science education of students. In addition to educating students about biotechnology, laboratory exercises in biotechnology techniques provide students with problem solving, inquiry-based, hands-on experiences that build confidence, demystify the subject, and make biotechnology relevant. To provide students with the skills needed to comprehend scientific advances and social implications of biotechnology, our students must be competent and confident in content knowledge and performance pedagogies for biotechnology applications.

Because of the relatively rapid pace of scientific advancements in biotechnology and molecular biology, many experienced middle and high school teachers have little or no formal training or hands-on experience in this content area. Three frequently used tools in plant biotechnology, which includes genomics and proteomics, are gel electrophoresis, polymerase chain reaction (PCR), and DNA fingerprinting. To incorporate these techniques into middle and high school and college curricula, content instruction and experience in laboratory techniques and safety procedures are required.

Electrophoresis basics

Electrophoresis can be simply and concisely defined as the movement of charged molecules toward an electrode of the opposite charge (Clark and Russell, 2005), and this movement can be used to separate molecules such as proteins, DNA, or other compounds in mixed samples on the basis of size or weight. The electrophoretic process can be conveniently thought of as an assembly of the following four elements: 1) buffer(s), 2) a solid phase or medium ("the gel"), 3) an electric current, and

Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
29,574	fl oz	µL	3.3814×10^{-5}
29.5735	fl oz	mL	0.0338
2.54	inch(es)	cm	0.3937
28.3495	oz	g	0.0353
28,350	oz	mg	3.5274×10^{-5}
1	ppm	$ng \cdot \mu L^{-1}$	1
(°F–32) ÷ 1.8	°F	°C	(1.8 × °C) + 32

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4) a way to visualize the molecules in the medium after electrophoresis. Most routinely performed electrophoresis protocols consist of a more or less standard set of these elements, although some variations are used for specialized and individualized applications.

Buffers

The charge on the molecules is influenced by the pH (a measure of acidity or alkalinity with 7 = neutral, <7 = acidic, and >7 = basic) of the solution and is typically controlled in electrophoresis with one or more of the following buffers: the loading buffer (the buffer in which the sample is contained), the gel buffer, or the running (electrode or reservoir) buffer. A buffer can be defined as a chemical system {e.g., a mixture of a weak acid and its salt or a single molecule, such as Tris base [Tris (hydroxymethyl)-aminomethane]} that prevents large changes in pH when hydrogen ions (acid) are added to or removed from the solution. The buffer solution may also contain other compounds, including a divalent cation chelator such as Na_2EDTA [(ethylenedinitrilo) tetraacetic acid disodium salt], especially when working with nucleic acids. Loading buffers for protein samples are often different from gel and running (reservoir or tank) buffers, whereas the gel and running buffers are almost always the same for typical DNA electrophoresis.

For the purposes of this discussion, we will limit our consideration to two of the following more common buffers used in nucleic acid electrophoresis and some other applications: Tris (base)-acetic acid-Na_2 EDTA (TAE) and Tris-boric acid-EDTA (TBE). Most of the buffer solutions, including theses, are made at concentrations of 50× or 10×, and require dilution with deionized water before use. These buffers serve to maintain the pH of the solution around 8.3. In addition to pH, the buffering capacity of the solution must be considered. The buffering capacity dictates the amount of hydrogen ions that can be added or eliminated from the solution while still maintaining the desired pH. Buffering capacity is generally a function of the concentration of the chemical moieties used to make the buffer (i.e., the greater the molarity of the buffering agents, the higher the buffering capacity). High buffering capacity is not always warranted or desirable. TAE and TBE are standard buffers that have sufficient buffering capacity for most applications. At their slightly basic or alkaline pH, these buffers permit most molecules in the samples to have a net negative charge. The molecules to be separated electrophoretically are placed near the black or negative (cathode) electrode so that in an electric field generated by direct current (DC), the molecules will move or migrate toward the red or positive (anode) pole. Note that some molecules, such as certain proteins and a few dyes, may carry a net positive charge in standard buffer systems and will migrate toward the negative electrode.

Two other important functions of buffers are related to conduction of electricity and dissipation of heat generated during the electrophoresis process. Pure water, in addition to being a very poor buffer, does not conduct electricity very well. The ions in the running buffer and the gel are essential for conducting the electric current between the two electrodes. How well electricity is conducted is related to the ionic strength of the buffer, which in turn is also related to buffering capacity discussed previously. However, the ionic strength of the buffer also affects the heat generated during electrophoresis. Generally, buffers with high ionic strength tend to generate excess heat. The reservoir buffer is also important for absorbing and dissipating some of the heat generated from the electrical resistance in the buffer and the gel. The tank buffer acts as a heat "reservoir" and keeps the gel relatively cool during electrophoresis. For a more complete discussion of buffers, including ionic strength and buffering capacity, see Andrews (1993).

Solid phase

The solid phase in electrophoretic applications usually consists of a gel, which is a solid evenly dispersed or suspended in a liquid that forms a more or less solid, but flexible, medium. The gelling medium can be made from a variety of materials, including potato starch, agarose, or acrylamide. In the past, gels made from potato starch were used for isozymes (protein) studies, but are seldom used today because of the inconsistency of preparations, and they have been replaced with gels made from agarose or acrylamide. Agarose is a highly purified form of agar and is used typically at concentrations of 1% to 3% (w/v). Agarose gels are used for separating nucleic acids (DNA and RNA) and other classes of molecules, but generally not for proteins. It produces a gel with fairly large and uniform pores through which molecules migrate in the electric field. These gels are prepared easily by mixing running buffer with the appropriate amount of agarose, melting the agarose with heat produced in a microwave or other source, and then pouring it as a "slab" or "horizontal" gel (parallel to the table surface). Agarose gels are easy to make and ideal for many teaching as well as research applications. Acrylamide gels, in contrast, are typically made from 5% to 12% (w/v) acrylamide, a synthetic repeating linear polymer, which is cross-linked together with a second type of acrylamide (N,N'–methylene-bis-acrylamide or piperazine diacrylamide). These types of gels are often referred to as polyacrylamide (two types of acrylamide) gels (PAG). Gel polymerization with PAG is accomplished using chemical accelerators (N,N,N', N'-tetra-methyl-ethylenediamine and 10% ammonium persulfate) in contrast to the simple melting of agarose with heat. Acrylamide gels are usually poured in vertical "slabs" (right angle to the table top) as a liquid, which typically requires 10 to 20 min to "gel" or they are used to fill the lumens of very thin tubes that are found in capillary electrophoresis. The higher the percentage of acrylamide, especially the cross-linker, the smaller the pores will be in the gel. PAG is used for separation of proteins and nucleic acids. However, acrylamide gels are more difficult to work with than agarose gels and contain some very toxic components. For example, unpolymerized acrylamide is a potent neurotoxin. All of the laboratories described in this article will be completed using agarose gels.

Regardless of the gelling agent used, the liquid portion of the gel will be 1× TAE or TBE. The sample molecules migrating in the electric field will move or migrate within the buffer contained in the pores of the gel. The higher the concentration of

gelling agent, the more "slowly" the substances will move through the gel and smaller differences (weight for proteins and base pairs for DNA) between the different samples will be detected by differences in migration distances. Therefore, in this context, acrylamide gels generally have better resolution (the ability to detect differences) than agarose gels.

Electric current

Because the electrophoretic process requires polarity (a negative and positive pole) for movement of the samples through the gel, DC provides the electromotive force that pushes the negatively charged particles through the gel toward the positive pole. A power supply transforms the alternating current (AC) from the electrical supply of the building to DC. The voltage employed is customarily between 50 and 300 V, although 100 V is used in many applications. The relative speed at which the molecules migrate in the gel is controlled primarily through the voltage. Higher voltages (>100 V) will cause the molecules to move more quickly through the medium, but will also produce excess heat, which may distort the final pattern or profile of the electrophoretically separated molecules. Some laboratories use higher voltages, but maintain the electrophoresis unit in a cool room or provide some way to circulate chilled buffer over the gel. Cooling gels can also present problems (Andrews, 1993), but are beyond the simple discussion presented herein. Therefore, in our experiments, we will use lower voltages and will not need additional cooling for the gels.

Visualization of molecules

There are a number of methods available to visualize target molecules in gels, but our discussion will be limited to protein and DNA detection. Of course, some molecules, such as food dyes that maintain their color throughout the electrophoresis, do not require an additional method for detection. DNA and protein molecules in acrylamide gels may be stained with various relatively complex procedures involving silver—the molecules appear as dark brown or black bands in the gels. Some of the staining processes require long periods of time and complicated protocols in addition to use of toxic reagents, such as formaldehyde. Proteins can also be detected using simpler methods with dyes (e.g., Coomassie blue among many others), which imparts a color to the molecules and creates bands, but these methods are less sensitive (not able to detect smaller quantities of the molecules) than silver staining methods.

DNA molecules separated in an agarose gel are easily visualized by color-inducing stains (e.g., methylene blue) or by fluorescent stains such as ethidium bromide or one of the SYBR® series of fluorescent stains (Applied Biosystems, Foster City, CA). The protocols used for these stains are very simple, but the stains are generally not very sensitive to small amounts of nucleic acids. Additionally, the fluorescent stains require an ultraviolet light source to be seen, and ethidium bromide is toxic, which presents potential health and disposal problems. Generally, silver staining can detect smaller quantities of DNA than can the other stains. Silver staining is most often used with acrylamide gels, which have better resolving power than agarose gels. Silver staining of DNA has the same advantages and disadvantages as do silver staining of proteins.

So now that all the elements of the electrophoresis have been described, we are ready to undertake two relatively simple experiments: electrophoresis of food dyes and electrophoresis of DNA after amplification with PCR. The experiment with food dyes requires minimal laboratory equipment and is an inexpensive and colorful exercise suitable for any group of students (and teachers) without experience with electrophoresis. The DNA exercise requires more complex equipment and reagents and is more expensive to complete.

The experiments are divided into "checklists" that contain all of the materials necessary to complete the exercise, "worksheets" that are explanations on how to make reagents or solve problems, and "procedure boxes" that include detailed instructions for completion of the laboratories. Although we have tried to provide exercises that minimize exposure to toxic substances, good laboratory safety should always be practiced. This includes, but is not limited to, no eating or drinking in the laboratory, no pipetting by mouth, wearing eye protection when necessary (melting of agarose), and absolutely no use of tobacco products of any kind. The equipment listed in the checklists is what we have used in our exercises, but there are other suitable alternatives.

Expt. 1. Electrophoresis of food dyes

This is a very simple experiment that is well suited to almost any age group—elementary school students as well as graduate students and teachers have enjoyed this exercise. The impetuous for its design was the curiosity of a middle school student asking the questions about an egg dying display in a grocery store, "Why are there so many different brands of food dyes?" and "Aren't they [the colors (e.g., red)] all the same?" The exercise is ideal for "electrophoresis novices"—it is an excellent means for illustrating the basic principles of electrophoresis and has never failed to perform as planned.

We have found that this exercise works best with groups of two to four students at each station. The materials needed for each group to complete the exercise can be found in Checklist 1. For the basic electrophoresis experiment, we suggest using food dyes from one manufacturer; you will need two suppliers to complete the "crime scene" dye exercise and for later exercises comparing colors from four different suppliers. Examples of suppliers that we have used include the following: Kroger Co. (Cincinnati, OH), McCormick & Co. (Hunt Valley, MD), Dudley's (distributed by The Paper Magic Group, Scranton, PA), and Paas (Signature Brands, LLC, Ocala, FL). There may be other suitable brands of food dyes that will work equally as well. Instructors are encouraged to evaluate the various dyes and select the ones that best suit your needs.

The first operation is to set-up the gel rig. Follow the instructions in Procedure Box 1 to assemble the gel rig. There are a number of different gel rigs available, but they all generally follow the same steps that we describe for assembling the apparatus and casting of the gel.

It is very important that the inner tray be level and seated properly

on the outer tray. The inner tray should be in the form of a "solid" rectangle and is now ready to receive the molten agarose. Follow the instructions outlined in Procedure Box 2 to dilute the 50× TAE stock buffer to make 1× TAE and to make the agarose gel.

Whether the laboratory exercise being taught deals with molecular biology, plant tissue culture, biotechnology, or chemistry, a requisite skill for students to learn is how to make dilutions. In our experience, instructors as well as students have difficulty with the concepts of dilution, including the algebra. At this juncture in the exercise, there is an excellent opportunity to review how to make dilutions (Worksheet 2). This is time well spent, as the second exercise (DNA Fingerprinting) will be more meaningful if students understand and have mastered dilutions.

Checklist 1. Materials needed per group of students for electrophoresis of food dyes.

- 2- to 20-µL and 10- to 100-µL pipettes and corresponding disposable tips (or other suitable device, such as a thin-tip transfer pipette (SAMCO Transfer Pipets, catalog no. 231; Samco Scientific Corp., San Fernando, CA; Fig. 1)
- Microwave oven
- Either a 500-mL or 1000-mL graduated cylinder and either a 125-mL or 250-mL Erlenmeyer flask
- Electrophoresis dye and crime scene kit (see Fig. 1 and Checklist 2).
- Ruler (centimeter), colored paper, and notebook
- Several paper towels or hot gloves and eye protection
- Gel apparatus with combs (Mini-Horizontal Unit with 6-place and 10-place combs; Fisher Scientific, Atlanta) and power supply (FB300; Fisher Scientific)
- Distilled water (distilled water from grocery store or if not available, tap water will work)
- White paper, food cling wrap and digital camera (optional)
- One 9-cm-diameter plastic petri dish for each student group and 30 mL of molten 1% microbiological grade water agar per petri dish.
- 125-mL flask of tap water
- Centrifuge tube of 100 µL of red dye in 60% glycerol

Procedure Box 1. Setting up the gel rig (apparatus).

- The inner box with two open ends needs to be positioned properly in the gel rig or outer tray. Rotate the inner box so that the orange rubber gaskets (open ends) are oriented to the sides of the gel rig. It is also helpful to moisten the gaskets lightly with water; this will help the apparatus to slide easily into the proper position. Note: Some older gel rigs require you to tap the sides. Please ask your teacher for instructions.
- Push the inner gel box downward into the outer gel box until it is seated snuggly on the bottom of the outer box. Be sure that the inner box is flat and level, and the orange gaskets are flat against the gel rig.

Procedure Box 2. Making a 1% (w/v) agarose gel.

- Open the 15-mL conical centrifuge tube in the dye and crime scene kit and pour the 10 mL of 50× TAE {Tris base [Tris (hydroxymethyl)-aminomethane]-acetic acid–Na_2EDTA [(ethylenedinitrilo) tetraacetic acid disodium salt]} buffer into a 500-mL or 1000-mL graduated cylinder. Carefully add distilled water (tap water will work if distilled water is not available) to make 500 mL of 1× TAE buffer. Swirl to mix. To see how this dilution was determined and to review how to make dilutions, consult Worksheet 2.
- This is the 1× TAE buffer used to make the gel and to fill the reservoirs in the gel rig. Pour 40 mL of 1× TAE buffer into the 250-mL (or 125-mL) Erlenmeyer flask, and then add 400 mg of agarose, which is contained in a small plastic bag. Swirl the contents.
- Microwave the suspension for about 1 min on high in the microwave to melt the agarose; do not let the suspension boil over.
- Remove the flask from the microwave using heat-resistant gloves. Inspect the contents of the flask to see if the agar has dissolved (remember always to wear eye protection and point the opening of the flask away from your face and others). If not, heat the suspension in the microwave for another 10 s and check again. Repeat microwave heating until all of the agarose is dissolved.
- The gel solution will appear clear when the agarose is completely melted.

Molecular biology-grade agarose and distilled water are specified as standard materials for this exercise. However, if these materials are not available, lower grade agarose or even microbiological-grade agar and tap water can be substituted without sacrificing illustration of the principles of electrophoresis. Lower grades of agarose and agar are less consistent and do not create a gel with uniform pore sizes. These media contain higher salt concentrations than molecular-grade agarose and this will influence the electrophoretic process. Tap water, depending on the source and location, will also add salts to the gel and the 1× TAE buffer. Water quality affects the process less than the quality of the gelling medium. Essentially, the dyes will move more slowly (less distance/time) in a 1% (w/v) microbiological agar gel and the bands will appear more diffuse than in agarose gels. Depending on the grade of agar available, sometimes a gel containing less than 1% agar (0.6%–0.9%) alleviates some of the problems discussed above. None-the-less, the dyes will migrate differentially through gels made with agar, albeit less clearly than in agarose.

In the development of this laboratory, we discovered that some dyes from suppliers not only were different from each other, but some individual dyes had multiple components. In other words, the red color from manufacturer A was composed of two different red dyes (bands in the gel) or contained another modifier color, such as blue, whereas another supplier had only one dye component (a single band). Differences in the composition of the dyes lend themselves to a mock "fingerprinting" or forensic exercise, which can be worked into a crime scene scenario. Trigiano and

Worksheet 1. How to make solutions for the experiments.

50× TAE buffer:

Under a fume hood, to 175 mL of distilled water, add:

- 60.5 g Tris base [Tris (hydroxymethyl)-aminomethane] (T),
- 14.3 mL of glacial acetic acid (A), and
- 25.0 mL of a solution containing:
- 18.6 g of Na_2EDTA [(ethylenedinitrilo) tetraacetic acid disodium salt] per 100 mL of distilled water (E).

Stir to dissolve, and then bring the total volume to 250 mL with distilled water and mix well.

The pH of the buffer should be about 8.3.

Food dye solutions:

- Add 40 mL of distilled water to 60 mL of glycerol to make a 60% (v/v) solution. Hint: Heat the glycerol to about 37 °C; it is much easier to measure the volume when warm.
- Dispense 5.0 mL of 60% glycerol into each of four 15-mL centrifuge tubes; one tube for each color.
- Add 5 drops of food dye to each tube (one color per each tube) and mix thoroughly. For dyes made from tablets, dissolve in a minimum of distilled water without vinegar. Add dye solution to the 60% glycerol until it is a dark color.
- For a mixed dye solution, combine 1.0 mL of each of the other four colors in a separate tube.
- Dispense about 20 µL of each color into 0.6-mL Eppendorf tubes.

Worksheet 2. Dilutions made simple.

In this example, we started with 10 mL of 50× TAE {Tris base [Tris (hydroxymethyl)-aminomethane]-glacial acetic acid–Na_2EDTA [(ethylenedinitrilo) tetraacetic acid disodium salt]} buffer and need to make 1× TAE buffer. The question is how much distilled water should be added to the original 10 mL of 50× buffer solution?

Use the following equation: $C1 \times V1 = C2 \times V2$,

(Where C1 is the initial concentration of the buffer, V1 is the volume in milliliters, C2 is the desired or final concentration of the buffer, and V2 is the final volume of buffer.)

Substitute known amounts: 50×10 mL $= 1 \times ?$ (V2 in milliliters)

Solve for ?: 50×10 mL$/1 = ? = 500$ mL (the final volume of water)

How much water to add: V2 – V1 = W = how much water is needed (remember we started with 10 mL of 50× TAE)

Substitute and solve for W: 500 mL – 10 mL = 490 mL of water.

Of course, the proper way to make the solution is to bring the volume in the graduate cylinder up to 500 mL with distilled water.

Worksheet 3. Preparation of "crime scene" dye evidence.

- Make hole punches (dots) from absorbent white paper.
- Place 10 µL of Kroger Co. (Cincinnati, OH) red dye on the "Crime Scene" and Suspect 2 dots.
- Place 10 µL of McCormick & Co. (Hunt Valley, MD) red dye on the "Suspect 1, 3, and 4" dots.
- Allow the food dyes to dry overnight and then place one dot in each of the appropriately labeled ("CS" or "S1," etc.) 1.5-mL Eppendorf tubes.

Note: If Kroger Co. and McCormick & Co. dyes are not available, investigate other supplier dyes. Two dyes of the same color (e.g., blue) with different bands are needed for this experiment.

Trigiano (2007) contains a "Who Done It" story that we have used with middle and high school students and in teacher workshops. This exercise can substitute for the DNA fingerprinting exercise (Expt. 2) and is useful for illustrating the concepts of DNA fingerprinting.

The crime scene samples are in centrifuge tubes, which are labeled S1 through S4 for the four suspects and crime scenes for the "evidence," and must be reconstituted. The samples are prepared inexpensively by using circular pieces (hole punches) of white, absorbent paper. Prepare the samples at least 1 day before they are needed by following the instructions outlined on Worksheet 3.

While the agarose in the flask is cooling, locate the "Crime Scene" kit (Fig. 1) mentioned in Checklist 2. Follow the instructions listed in Procedure Box 3 to prepare the crime scene samples. Note: This part of the exercise may be omitted if only working with food dyes.

The agarose should be cooled sufficiently to pour into the inner tray. Follow the instructions in Procedure Box 4 to cast the gel. Pay close attention so that bubbles are not present in the gel or around the combs.

Observe the gel as it "solidifies"—it should turn from clear to somewhat opaque. After about 20 min, the gel should set to the final configuration. Complete the instructions listed in Procedure Box 5. Note: For those with limited time in each class/laboratory period, this is a convenient stopping place. Cover the gel with plastic cling wrap or place it inside a resealable plastic container with a few moist paper towels. The gel may be stored in the refrigerator at 4 °C for 1 week or more. Also, cover the container of 1× TAE buffer to prevent evaporation.

The gel is now ready to receive the dye solutions—be sure that 1× TAE buffer has been added to the reservoirs and covers the gel surface and that the wells are positioned closest to the negative (black) electrode. It is important not to overload the wells; only about 3 to 5 µL are

Fig. 1. Components of the food dye and crime scene dye kit.

Checklist 2. Contents of dye and crime scene kit.

- A plastic, conical 15-mL centrifuge tube containing 10 mL of 50× TAE {Tris base [Tris (hydroxymethyl)-aminomethane]-glacial acetic acid—Na_2EDTA [(ethylenedinitrilo) tetraacetic acid disodium salt]} buffer.
- A 1.5-mL Eppendorf tube containing about 1 mL of 60% glycerol
- A small plastic bag containing 400 mg of molecular grade agarose–a white powder–sufficient to make 40 mL of 1% (w/v) agarose gel.
- A plastic bag labeled of "food dyes" including red, blue, green, and yellow and a mixture of colors from one manufacturer contained in 0.6-mL Eppendorf tubes. This experiment was developed around Kroger Co. (Cincinnati, OH) food dyes. An alternative experiment is to prepare food dyes from a variety of manufacturers and compare the various colors. The alternative experiment included the dyes supplies provided above and dyes from the following brands: McCormick & Co. (Hunt Valley, MD), Paas (Signature Brands, LLC, Ocala, FL), and Dudley's (distributed by The Paper Magic Group, Scranton, PA). The alternative experiments are depicted in Trigiano and Trigiano (2007).
- Preparation of food dyes, 60% glycerol and 50× TAE are described in Worksheet 1.
- A small plastic bag containing five 1.5-mL Eppendorf tubes labeled "Crime Scene." See Worksheet 3 for preparation of crime scene materials.

Procedure Box 3. Preparing the "crime scene evidence."

- Open the bag labeled "Crime Scene" and remove the five 1.5-mL Eppendorf tubes labeled "CS," "S1," "S2," "S3," and "S4"–"CS" represents the evidence gathered from the "crime scene" and "S1–4" represents evidence gathered from four "suspects."
- Add about 100 µL of 60% glycerol solution to each of the five tubes in the "Crime Scene" kit using one of the transfer pipettes in the kit. Be sure that the paper disks in the tubes are immersed in the glycerol solution. Set aside for about 20 min.

Procedure Box 4. Casting the agarose gel.

- Mix the cooled, but not hardened, agarose by swirling and without introducing air bubbles, and then pour it into the inner gel box.
- Remove any bubbles in the solution by raking the gel solution with the teeth of the comb. Insert the comb (10 thickest teeth) into the slot 2.5 cm from the side of the gel rig. Note that two replications of the dyes only can be completed if the crime scene has not being used.
- Allow the agarose solution to cool and harden (gel) for about 20 min.

Procedure Box 5. Setting up the gel for electrophoresis.

- After the gel has hardened (appears opaque or cloudy), pull the inner box out of the gel rig. Be careful not to disturb the comb.
- Rotate the inner box containing the gel 90° and insert the inner gel box into the outer gel box with the combs closest to the black electrode. Caution: Try to maintain the inner gel box level or parallel to the work station. If the inner gel box is tilted, there is a possibility that the gel will slip from the box and fall on the table.
- Pour the 1× TAE {Tris base [Tris (hydroxymethyl)-aminomethane]-acetic acid–Na_2EDTA [(ethylenedinitrilo) tetraacetic acid disodium salt]} buffer into the reservoirs of the gel box and over the gel to a depth of 1.5 cm. There should be some 1× TAE buffer remaining.
- Gently remove the comb from the gel by pulling it straight upward.

necessary for good color development (for a representation, see Fig. 2). Follow the instructions outlined in Procedure Box 6 to load the gels. This part of the exercise is perhaps the most difficult for students and when they will need the most help. At the end of Procedure Box 6, there is a supplementary exercise that allows students to practice "loading gels" in petri dishes instead of the gel apparatus.

It is inevitable that some of the dye solution will not make it into the wells and will settle on the surface of the gel, especially if you are using the small transfer pipettes. This is not a problem; only the dyes in the wells will enter the gel matrix. However, try to begin the electrophoresis as soon as possible after the dyes have been loaded. Follow the instructions listed in Procedure Box 7 to complete this part of the exercise.

After 35 min, turn off the power supply and remove the gel. If available, use a digital camera to photograph the gels for a permanent record. Students should also make a drawing of the gel in their notebooks (Fig. 3) using the instructions provided in Worksheet 4. The worksheet provides practice for simple ratios and algebra.

If conducted according to the instructions provided and if the dye and crime kits (Fig. 1) are prepared, the entire exercise should take 1 to 1.5 h to complete. An alternative to Expt. 1 is to compare similar colored dyes from different manufacturers using the same protocols as described previously. The color components of some of the dyes will surprise the students (Fig. 3). Sometimes it will be necessary to use a 2% gel [w/v (800 mg agarose per 40 mL of 1× TAE buffer)] to visualize some of the "minor" components of a dye. For example, many times, the blue and yellow dyes may contain a small proportion of red dye. The results from such experiments are shown in Trigiano and Trigiano (2007).

EXPT. 1. Questions for thought and review (answers can be found at Trigiano and Trigiano (2007)).

1. How might the dye bands appear after 1 h of electrophoresis?
2. How would the dye bands appear in a 3% (w/v) agarose gel after 35 min of electrophoresis?
3. Would the dyes migrate at the same rate and direction if a much

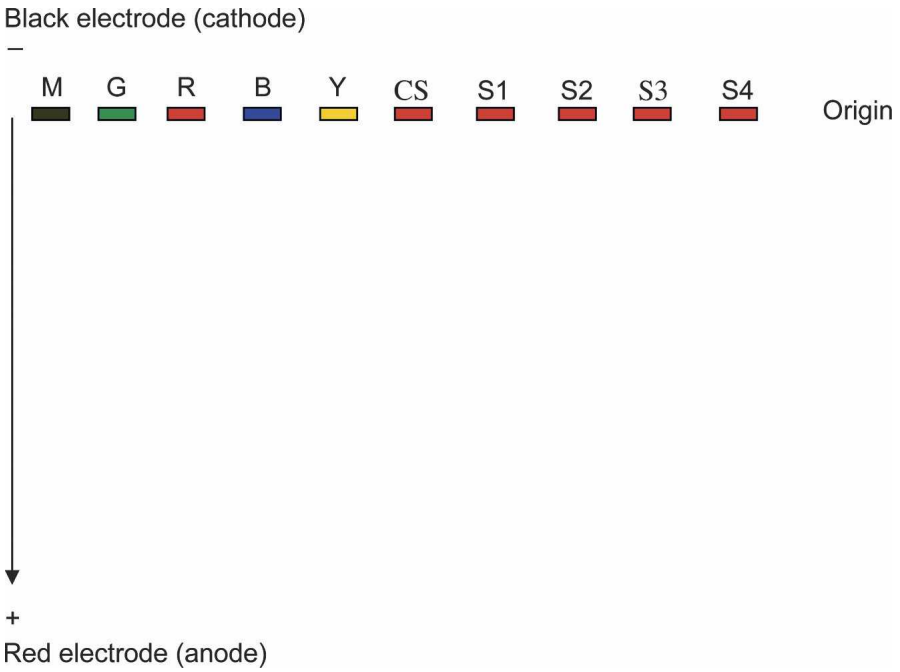

Fig. 2. Graphic representation of loading the gel with food dyes and crime scene evidence before electrophoresis (M = mixed colors; G = green; R = red; B = blue; Y = yellow; CS = crime scene evidence; S1–4 = suspects). All food dyes in the lanes were prepared from Kroger and Co. (Cincinnati, OH) products except for those in lanes S1, S3, and S4, which were prepared with food dyes from McCormick & Co. (Hunt Valley, MD).

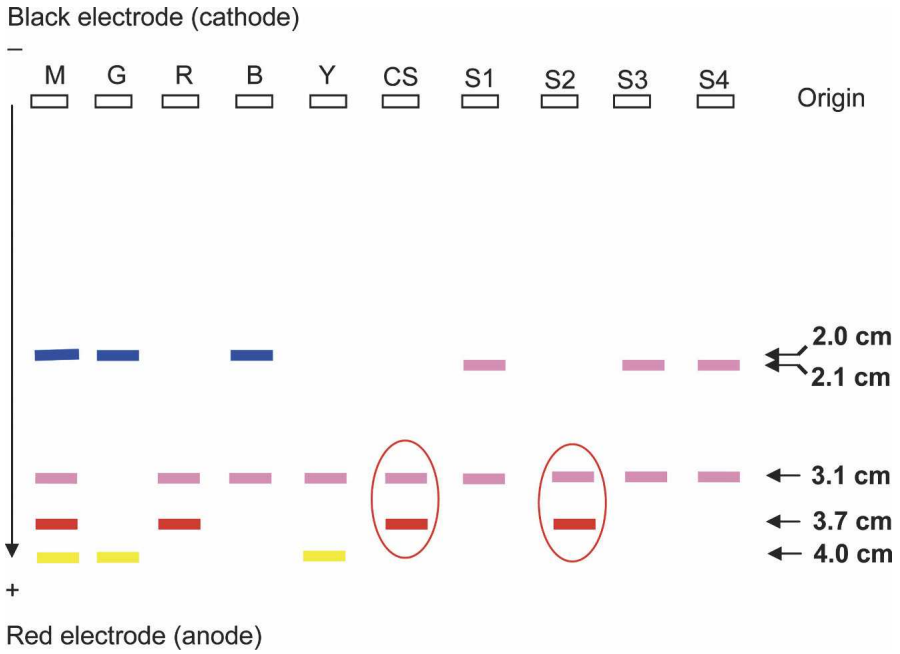

Fig. 3. Graphic representation of food dyes and crime scene evidence after (35 min) electrophoresis (M = mixed colors; G = green; R = red; B = blue; Y = yellow; CS = crime scene evidence; S1–4 = suspects). Circled bands in the CS lane match circled bands in the S2 lane. Compare with lanes S1, S3, and S4. All food dyes in the lanes were prepared from Kroger and Co. (Cincinnati, OH) red dye except for those in lanes S1, S3, and S4, which were prepared with food dyes from McCormick & Co. (Hunt Valley, MD); 1 cm = 0.3937 inch.

lower pH buffer instead of a buffer at 8.3 were used to make the gel?

4. You suspect that a dye is made of one major color and a very minor color, but the minor color cannot be seen in 1% (w/v) agarose gel loaded with 3 to 5 µL of dye. What could you do to detect the minor dye?

5. How would the migration of the dyes be affected using 25, 50, or 200 V instead of the more typical 100 V?

6. What characteristic of molecules (food dye, DNA, proteins, etc.) allow the molecules to migrate differentially in a gel (agarose, acrylamide, etc.) matrix during electrophoresis?

7. What roles do buffers play in electrophoresis?

8. When DNA molecules are separated by electrophoresis, why is a "staining step" necessary?

9. Why do we make the wells in an agarose gel on the end of the gel rig closest to the negative (black) electrode?

Expt. 2. DNA amplification of the internally transcribed spacer (ITS) region

As a society, we are constantly bombarded with references to DNA testing by television dramas, news reports, and printed material such as newspapers, periodicals, textbooks, and novels. Of course, what they are all referring to is essentially PCR, which makes the characterization of DNA possible. Whether we want to know the paternity of a child, the phylogenic relationship between species within a genus of plants, or the sequence of a specific gene, we use some variation of PCR. As such, our students need to understand and appreciate the basics of this very important and powerful technology. This experiment is designed to give students hands-on experience with PCR.

The instructor may elect to allow participants to isolate DNA, make the reagents, set-up the PCR reactions, and/or run the gels. For DNA isolation, there are a number of commercially available kits (e.g., Puregene, Gentra Systems, Minneapolis, MN; and Qiagen, Valencia, CA) available that provide adequate quantity and quality of DNA for the reactions. Many of the suppliers of the materials

Procedure Box 6. Loading the food dyes and "crime scene evidence."

- Remove the food dyes from the plastic bag and centrifuge at low speed so that the dye solutions migrate to the bottom of the tubes. If a centrifuge is not available, tap the bottom of the tube on the table. Most of the solutions should collect in the bottom of the tube.
- Mix the crime scene dye tubes by gently "flicking" them with your finger, then centrifuge as you did for the food dyes. Note: The crime scene dye solutions should appear very red after 20 to 30 min.
- Choose an order to load the food dyes (e.g., lane 1 = Yellow, lane 2 = Red … and lane 5 = Mixed) and record in your notebook. Lane 6 = CS, lanes 7–10 = Suspects 1–4, respectively. Refer to Fig. 2 for a typical loading arrangement.
- Adjust the volume of the pipette to 3.5 μL and load the first color into the first well of the gel. Alternatively, use a transfer pipette to load the gel. Gently squeeze the bulb, insert the tip into the solution, and fill the first "joint" of a transfer pipette with dye (see Trigiano and Trigiano, 2007). With a little practice, this is a very satisfactory and inexpensive alternative to loading the gels with micropipettes. Do not stab the gel, but place the tip of the pipette either into or slightly above the well and GENTLY dispense the dye solution. The glycerol containing the dye will sink to the bottom of the well. There is no need to change tips or use additional transfer pipettes. Very little of the previous dye will contaminate the next sample; agarose gels do not have the resolution to detect this small quantity of dye. Load the remaining dye solutions.
- NOTE: It may be helpful to place a piece of colored paper under the gel apparatus. This helps to give some depth of field and aids in loading the dyes. Most students find it relatively difficult to load the first well with dye, but subsequent wells are easier to load because the first dye provides a spatial reference. Some, perhaps most, students are a little uncomfortable with the idea and mechanics of loading gels. A simple practice loading exercise is to pour 1% water agar (microbiological grade) into plastic 9-cm-diameter petri dishes, place combs in the molten, but cooled agar, and allow the gel to harden. After the gel has hardened, pour tap water (simulates buffer) into the petri dish and remove the combs. Allow the students to practice loading the petri dish gel using the micropipettes or the transfer pipettes and the centrifuge tube containing the 100 μL of red dye. The petri dish gels can be prepared in advance of the actual laboratory exercise to save time. This exercise is depicted in the PowerPoint™ (Microsoft Corp., Redmond, WA) presentation (Trigiano and Trigiano, 2007).

Procedure Box 7. Conducting the electrophoresis.

- Place the lid on electrophoresis box and connect the electrodes to the direct current (DC) power pack (black to black and red to red). Adjust voltage to 100 V.
- Run for 25 to 35 min and then turn off the power supply. Note: 35 min run time gives superior resolution of the dyes.
- Remove the inner tray from gel rig, drain the 1× TAE {Tris base [Tris (hydroxymethyl) aminomethane]-glacial acetic acid—Na_2EDTA [(ethylenedinitrilo) tetraacetic acid disodium salt]} buffer, and place the gel on a sheet of white paper or food cling wrap.
- Collect data using the digital camera. Using a ruler, measure in centimeters how far each dye has migrated through the gel (distance between well and the middle of the dye front in centimeters). Note: Typically, the distance moved is based on the dye front. However, in this experiment, the front often appears very "diffuse," but the middle part of the dye mass appears "solid." Diagram and record in your laboratory notebook the final dye positions (centimeters) as seen in the gel after electrophoresis (Fig. 3). Note: If you plan to have students write reports or prepare PowerPoint™ (Microsoft Corp.) presentations, it will be necessary to complete some simple math (proportions) to accurately represent the results of the laboratory exercise. Refer to Worksheet 4 for the method. Also note: These gels cannot be stored–the dyes are relatively small molecules and as soon as the electrophoresis is completed, the colors in the bands will begin to diffuse immediately. After a few hours, individual bands are no longer discernible.
- Clean equipment for the next run.
- Repeat procedure for each color or mixture of dyes.

may be found in Trigiano and Caetano-Anollés (1998) and Trigiano et al., (2007). However, we suggest that the instructor isolate DNA from the organisms or obtain it from a generous coworker. Furthermore, the exercise works well if the instructor makes all of the reagents and assembles the DNA kits for the amplification of the ITS region of the 5.8-ribosomal subunit (Trigiano et al., 2007). The DNA kit as described in this exercise should assure success; we have had over 100 participants ranging from middle school students to college professors complete this experiment and about 60% to 70% of the teams have successfully completed the exercise. We believe that the 30% to 40% failure rate is not due to flaws in the kit design, but rather because of uncontrollable factors such as participants not following or misreading instructions, the incomplete mixing of reagents, pipetting errors (especially with water, the only reagent provided in excess quantity), and/or participant inexperience. Even with this failure rate, many teams get results and can share them with the rest of the class.

The central idea of PCR is to make billions of copies of selected regions of DNA so that it can be visualized, sequenced, or inserted into a vector, to name a few applications. PCR reactions typically involve the following components: template DNA, which is isolated from an organism of interest; a buffer with salt [usually potassium chloride (KCl)] that maintains pH and ionic strength of the reaction; nucleotides in the form of adenine triphosphate (ATP), cytosine triphosphate (CTP), guanine triphosphate (GTP), and thymine triphosphate (TTP), which are the "building blocks" of the new DNA strands; primers, relatively small (8–25 bp) of DNA that dictate which regions of the template DNA will be copied; DNA polymerase, a thermostable enzyme that copies the DNA; and magnesium chloride ($MgCl_2$), of which the Mg^{+2} cation is a cofactor for DNA polymerase—the enzyme will not be active without this ion. The experiment will not describe the details of PCR—a downloadable educational resource provided by BioRad Laboratories (2004) depicts this process. This animation and another are included in Trigiano and Trigiano

Worksheet 4. Converting data from the gel to notebook or PowerPoint™ (Microsoft Corp., Redmond, WA) presentations.

- Draw the "fastest" moving yellow bands (4.0 cm) in the gel at 12.0 cm in the notebook or on the computer screen.
- To find out where to draw the slower moving red bands (2.7 cm in the gel), use the equation (proportions or ratios):

$$\frac{4.0\,\text{cm}}{12.0\,\text{cm}} = \frac{2.7\,\text{cm}}{?\,\text{cm}}$$ (in the notebook or on the screen)

- Cross multiply: 4.0 cm × ? cm = 12.0 cm × 2.7 cm
- Solve for ?: ? = 32.4 cm/4.0 cm or
- 8.1 cm from the origin.
- Draw the red bands at 8.1 cm in notebooks or on the screen.

Checklist 3. Materials needed for the DNA amplification and fingerprinting experiment.

- DNA amplification kit–see Worksheet 6 for components
- 10-µL and 100-µL micropipettes and corresponding sterile tips
- Gel apparatus with six-place comb and power supply
- 500-mL or 1000-mL graduated cylinder
- CarolinaBlu™ stain (Carolina Biological Supply Co., Burlington, NC)
- Microwave oven
- 125-mL or 250-mL Erlenmeyer flask
- Heat-tolerant (hot) gloves
- Distilled water
- Acetonitrile gloves
- Thermalcycler

(2007). The students will gain hands-on experience in pipetting, assembling master reaction mixtures, and staining DNA in agarose gels. For convenience and interest, the exercise is formulated as a crime scene. Instructors and students are encouraged to seek additional expertise on their campuses when needed.

Each team or group of students will require the items found in Checklist 3 to complete the exercise.

The success of the exercise depends on the use of the DNA kit. Each kit supplies all of the essentials for the experiment. In our case, there is one tube of crime scene DNA, and four different tubes (S1–S4) of suspect DNA, for a total of five samples. The assembly (calculation of volumes of components) of the master mix is based on six samples, to allow for pipetting errors, and is shown in Worksheet 5. The composition of the DNA kit is shown in Worksheet 6.

The kit is designed to minimize pipetting errors, thus maximizing the probability of successful amplification. However, be aware that students will still make some pipetting errors that may adversely affect the outcome of the PCR reaction. Follow the instructions listed in Procedure Box 8 to make master mixes and complete the PCR process.

The thermalcycler should be programmed as follows:

Step 1: 96 °C for 1 min—Denatures DNA

Step 2: 56 °C for 1 min—Primers anneal to ITS sites

Step 3: 72 °C for 1 min—Extension

Steps 1 to 3 should be repeated for 35 cycles

Step 4: 72 °C for 7 min

Step 5: 4 °C—Hold

Loading and running the gel is very similar to that described for the dye experiment, with a few exceptions. Follow the instructions listed in Procedure Boxes 1, 2, 4, and 5 to make the agarose gel. Remember to use the comb with the widest six teeth for this experiment. The DNA in the gel will need to be stained as well. Follow the instructions in Procedure Box 9 to complete this experiment.

At the end of the electrophoresis, two color bands should be visible; these bands are the tracking dyes, not the DNA (Fig. 4). Methylene blue will stain the DNA, but unfortunately, it will also impart some color to the gel matrix. Those with exceptionally good eyesight may be able to see the bands after the CarolinaBlu™ (Carolina Biological Supply, Burlington, NC) is removed, but typically, the gel must be destained for about 1 h before the bands are easily visible. Soaking the gels overnight removes almost all of the stain in the gel, but not bound to the DNA. For more advanced classes, instructors may choose to stain the DNA with ethidium bromide that had been incorporated into the gel before casting. This stain is toxic and requires an ultraviolet light source for visualization.

The slowest band [black fly (*Simulium tuberosum*) DNA], or the band that migrated the least distance, is the heaviest at about 1000 bp, and the fastest band (the fungus, *Sclerotinia homeocarpa*), or the band that migrated the most distance, is

Worksheet 5. Components of the DNA amplification master reaction mixture.

Reagent (concn)	Water	Buffer (10x)	Primer 1 (30 µM)	Primer 2 (30 µM)	Nucleotides (2 mM)	$MgCl_2$[z] (25 mM)	Enzyme
			Volume (µL)				
Each sample	6.2	2	2	2	2	1.2	0.6
Samples +1	6	6	6	6	6	6	6
Total volume (µL)	37.2	12	12	12	12	7.2	3.6

Magnesium chloride.

[z]Magnesium chloride.

Worksheet 6. Components of DNA amplification kits.

- Plastic bag containing 400 mg of molecular grade agarose and a 15-mL centrifuge tube containing 10 mL of 50× TAE {Tris base [Tris (hydroxymethyl)-aminomethane]-glacial acetic acid Na_2EDTA [(ethylenedinitrilo) tetraacetic acid disodium salt]} buffer
- Table top centrifuge
- A plastic box containing the following:
  - 0.6-mL Eppendorf tubes (instructor should prepare a kit using sterile tubes for each team of students and store at –20 °C until needed),
  - Tube "S1" with 4 µL of 0.5 ng·µL^{-1} fungal DNA – isolated by instructor (we used *Sclerotinia homeocarpa*),
  - Tube "S2" with 4 µL of 0.5 ng·µL^{-1} insect DNA – isolated by instructor [we used black fly (*Simulium tuberosum*)],
  - Tube "S3" with 4 µL of 0.5 ng·µL^{-1} plant DNA,
  - Tube "S4" with 4 µL of 0.5 ng·µL^{-1} insect DNA,
  - Tube "EZ" with 3.6 µL of DNA polymerase (Stoffel Fragment; Applied Biosystems, Branchburg, NJ),
  - Tube "NU" with 12 µL of 2 mM master mix of nucleotides (Bioline, Randolph, MA), and
  - Tube "P1" with 12 µL of 30 µM forward primer (5′-3′):

TCCGTAGGTGAACCTGCGG (ITS1; White et al., 1990; Integrated DNA Technologies, Coralville, IA). Primer DNA is received as a dry power (may not be visible) in a small tube.

To prepare a 300 µM stock primer solution, solve the following equation:

nanomoles of oligo (on manufacturer sheet)/X µL = 300 µM

e.g., 169 nmol/X µL water = 300 µM

X = 563 µL of sterile, distilled water.

Centrifuge the tube to settle the powder, and then add the water. Heat to 65 °C to help dissolve the DNA. Now dilute (1:9) 30 µL of the 300 µM stock primer with 270 µL of sterile, distilled water and mix well. This is the 30 µM primer solution to use in the PCR reaction. Store frozen (–20 °C) until needed.

  - Tube "P2" with 12 µL of 30 µM reverse primer (5′-3′): TCCTCCGCTTATTGATATGC (ITS4; White et al., 1990),
  - Tube "BU" with 12 µL of 10 X buffer – supplied with DNA polymerase enzyme,
  - Tube "MG" with 7.2 µL of 25 mM $MgCl_2$ (magnesium chloride) – supplied with DNA polymerase enzyme,
  - Tube "H_2O" with 100 µL of sterile, distilled water
- Tube "6×" with 30 µL of 6× green loading buffer (25 mg of orange G and 25 mg of xylene cyanol FF I in 10 mL of 30% glycerol). Store in refrigerator (4 °C). Note: 15 g of Ficoll 400 (Pharmacia, Piscataway, NJ) may be substituted for 30% glycerol and this solution does not need to be refrigerated.

Procedure Box 8. Making master reaction mixes for DNA amplification.

- Wear acetonitrile gloves. Centrifuge the contents of all of the tubes to assure that materials are on the bottom of the tubes. If a centrifuge is not available, gently tap the bottom of the tube against a hard surface.
- Adjust the 100-µL micropipette to 37.2 µL and aseptically add tip to the end of the pipette. Draw 37.2 µL of sterile distilled water into the tip and dispense into the tube labeled "BU." Close lid of the tube and mix by flicking.
- The same tip may be used again if not contaminated (has not touched any other surface); if so, change the tip. Adjust the 20-µL micropipette to 15 µL and transfer the contents of tubes "P1," "P2," and "NU" to the "BU" tube containing water and buffer. Note: These tubes contain 12 µL of each of the materials; the pipette was adjusted to 15 µL to assure complete transfer of the fluid. Close the lid of the tube and mix the contents by flicking the tube with your finger. If the students have difficulty with pipetting, the entire contents of the "BU" tube may be transferred to the "P1" tube, and in turn, transfer the contents to the next reaction constituent, including "MG," in the next step. This alternative is depicted in Trigiano and Trigiano (2007). Note: The alternative procedure works well with transfer pipettes if micropipettes are not available.
- Adjust the 20-µL pipette to 9 µL and transfer the contents of the "MG" tube to the "BU" tube. Mix again.
- Adjust the 100-µL micropipette to 100 µL and draw the entire contents of the "BU" tube into the tip and transfer to the tube labeled "EZ." This is the master mix and constitutes the reaction mixture without the DNA templates. Close the lid and mix well. Centrifuge the tube to bring the contents to the bottom.
- Adjust the 20-µL micropipette to 16 µL and transfer 16 µL of the master mix in "EZ" to each of the following tubes: "CS," "S1," "S2," "S3," and "S4." Note: If using the plastic transfer pipettes, dispense the master mix equally into the five tubes. In our experience, the reactions will still work under these conditions. If using micropipettes, some fluid should remain in the "EZ" tube after this operation because sufficient master mix was prepared for six reactions. The total volume in the sample tubes is now 20 µL because each DNA template was contained in 4 µL. The reaction mixtures are now complete. Mix and centrifuge the five sample tubes and tightly close the lids. Label the tubes with a mark (symbol such as heart, star, etc.) that identifies your laboratory team. Place the tubes in thermalcycler and start the program. When the program is completed, the samples may be used immediately or stored in the refrigerator (4 °C) until needed.

about 500 bp. The band at an intermediate distance is from flowering dogwood (*Cornus florida*) at about 700 bp. The "crime scene: CS" DNA matches that of "Suspect 3: S3"—both DNA samples were from flowering dogwood. If insect DNA is unavailable, DNA from other sources may be used. Instructors should note that many fungal ITS regions are about 500 to 550 bp and many plant ITS regions are 700 to

Procedure Box 9. Electrophoresis and staining of DNA in agarose gels.

- Remove the sample tubes from the thermalcycler or refrigerator and centrifuge. Set the 20-µL micropipette to 4 µL and transfer 4 µL of loading buffer to each of the five sample tubes. Mix well and centrifuge again.
- In your notebook, draw the gel and assign a sample to each of the wells. Note: We suggest that the well closest to the black electrode (on the left side) remain blank. This will serve as a position reference. Set the 20-µL micropipette to 15 µL and transfer 15 µL of the samples (one per well) according to assignments in your notebook. There is no need to change tips between samples, as the sensitivity of the methylene blue DNA staining technique will not reveal minor contaminations. If silver staining were used, then changing tips would be advisable. Note: The plastic transfer pipettes can be used to fill the wells.
- Replace the gel rig lid, adjust the voltage to 100 V, and allow it to run for 1 h.
- Remove the gel from the apparatus, place in a tray, and cover with CarolinaBlu™ (Carolina Biological Supplies, Burlington, NC) for about 15 min. The gel will be stained dark blue.
- Pour the CarolinaBlu™ back into the original bottle (can be reused) and soak the gel in distilled water. Change the water frequently; bands of DNA should be evident within 1 h (Fig. 4). Washing overnight will destain the gel almost completely and DNA bands will be easily seen.
- Compare the distances the different DNA bands have migrated and find the two bands that appear to be identical or most similar. Case solved!

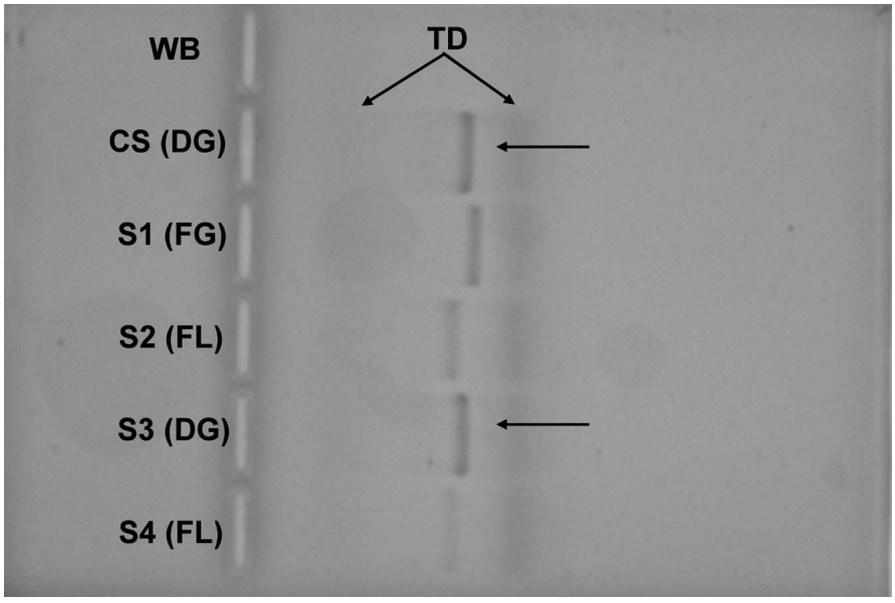

Fig. 4. DNA crime scene agarose gel stained with CarolinaBlu™ (methylene blue; Carolina Biological Supply). Electrophoresis was for ≈1 h. WB = blank well (if available, a molecular weight ladder may be placed in this lane); CS = Crime Scene DNA; S1–4 = Suspect 1–4 DNA; DG = plant DNA [flowering dogwood (*Cornus florida*)]; FG = fungal DNA (*Sclerotinia homeocarpa*); FL = insect DNA [black fly (*Simulium tuberosum*)]; TD = tracking dyes. The black fly DNA is the heaviest [≈1000 bps (bp)], the intermediate-sized band is the flowering dogwood DNA (≈700 bp), and the lightest band is the DNA from the fungus (≈500 bp). The tracking dyes are orange G (yellow in gel), which comigrates with DNA weighing ≈50 to 100 bp, and xylene cyanol FF (blue in gel and closest to the wells or the origin), which comigrates with DNA weighing ≈4000 bp. The arrows indicate a match between CS DNA and S3 DNA, based on similar electrophoretic mobilities of the DNA molecules in the gel.

800 bp. For most applications, DNA from species belonging to two different kingdoms will suffice for the experiment.

Expt. 2. Questions for thought and review [answers can be found at Trigiano and Trigiano (2007)].

1. Why is $MgCl_2$ needed in the PCR reaction? Can calcium chloride ($CaCl_2$) or manganese chloride ($MnCl_2$) be substituted for $MgCl_2$?
2. Why is a "master mix" made instead of assembling all of the reaction cocktails separately?
3. What would happen if the annealing temperature used in the thermalcycler program was decreased from 56 to 30 °C? Increased from 56 to 61 °C?
4. What is a chelating agent and why is it included in many buffers used in nucleic acid work?
5. Why are two primers necessary for the PCR reaction?
6. Although this DNA experiment did not include controls for simplicity, what controls should be included in the exercise?

Concluding remarks

The two experiments in this article are easy and relatively inexpensive for most laboratories to complete. The exercises are adaptable to almost any learning level and have a number of variations to suit almost any audience. The procedures require minimal equipment and knowledge, yet allows participants to gain an appreciation for electrophoresis, PCR, and DNA fingerprinting, which should help them to understand, albeit not completely, some of the science behind today's now common sensational news headlines. We estimate that the dye experiment costs about $0.25 to $0.50 per student, whereas the PCR experiment is more expensive at $0.75 to $1.50 per student, depending on the number of students on each team and in the classes. The costs do not include major equipment such as micropipettes, thermalcyclers, centrifuges, and freezers, or DNA isolation. However, most of the major equipment can be borrowed, and many laboratories will gladly provide DNA for your use. Additional resources and ideas for experiments are available at Trigiano and Trigiano (2007).

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