Laboratory Exercises

Inexpensive and Safe DNA Gel Electrophoresis Using Household Materials

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S. Ens,* A. B. Olson,† C. Dudley,‡ N. D. Ross III,* A. A. Siddiqi,* K. M. Umoh,* and M. A. Schneegurt,§¶

From *Northeast Magnet High School, Wichita Public Schools, Wichita, Kansas, †El Dorado Middle School, El Dorado Public Schools, El Dorado, Kansas, ‡East High School, Wichita Public Schools, Wichita, Kansas, §Department of Biological Sciences, Wichita State University, Wichita, Kansas

Gel electrophoresis is the single most important molecular biology technique and it is central to life sciences research, but it is often too expensive for the secondary science classroom or homeschoolers. A simple safe low-cost procedure is described here that uses household materials to construct and run DNA gel electrophoresis. Plastic containers are fitted with aluminum foil electrodes and 9-V batteries to run food-grade agar-agar gels using aquarium pH buffers and then stained with gentian violet. This activity was tested in a high school biology classroom with significantly positive responses on postactivity reflective surveys. The electrophoresis activity addresses several Life Science Content Standard C criteria, including aspects of cell biology, genetics, and evolution. It also can be used to teach aspects of motion and force in the physical science classroom.

Keywords: Molecular biology, high school, laboratory exercises, physical sciences.

INTRODUCTION

It is hard to imagine biology research without molecular biology techniques. Most biologists today rely on common molecular techniques such as agarose gel electrophoresis, polymerase chain reaction (PCR), and restriction enzyme digests to manipulate and study DNA molecules [1]. Although these techniques are widespread, the equipment and materials needed for their performance are invariably expensive, often out of the reach of secondary science classrooms and homeschoolers. In addition, some of the reagents are too hazardous for these settings.

Here, we report on gel electrophoresis systems that can be constructed and run using only safe household materials widely available from national hardware, pet supply, pharmacy, and supermarket chains. Previously reported alternative electrophoretic systems typically use a mixture of household and laboratory materials [2–5]. Although the quality of DNA gel electrophoresis and visualization using safe household materials does not rival that reached with research-quality materials, the gels are relatively inexpensive, easy to perform, and instructive in the secondary science classroom and for homeschooled

students. Electrophoresis can be combined with detergent-based DNA preparations [6, 7]; however, much of the flocculated material obtained is not DNA and often does not give good bands after electrophoresis.

National Science Education Standards [8] include the teaching of molecular genetics with an emphasis on the chemical nature of DNA. Electrophoresis activities address several life science standards presented here, as well as physical science standards discussed in a later section. Within Life Science Standard C, the activity addresses standards in The Cell (#1-4, 6), Molecular Basis for Heredity (#1-3), and Biological Evolution (#1, 4, 5). Through these investigations, with teacher guidance, students gain a deeper understanding of molecular genetics. Observing the molecular nature of DNA and discussing its roles demonstrates that specific components of cells underlie specific functions and that chemical reactions are important for maintaining cell components. Observing differences between DNA extracts from various organisms, leads to a discussion of mutation, evolution of species, common ancestors, and the balance between unity and diversity in living systems through evolutionary time. Physical separation and visualization of DNA fragments with this protocol should make these abstract concepts more real for students promoting better comprehension of the academic language of the discipline.

Our study was performed at Northeast Magnet High School (NEM) in Wichita, as part of an introductory biology course required of freshmen and sophomores.

[¶]To whom correspondence should be addressed. Email: mark.schneegurt@wichita.edu.

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Wichita Public Schools (WPS) is a majority–minority district of over 50,000 students in the largest city in Kansas. At NEM, the population of 600 students is 62% minority, with 51% economically disadvantaged. This is a science and technology magnet school, and only 15% of students scored below the acceptable level on 11th grade science assessments, half the district average. Before the activity, students need to have some understanding of the chemical nature of DNA, particularly that DNA molecules are polymers of different lengths determined by the number of base pairs they contain and that the DNA backbone contains phosphate groups that are highly negatively charged and thus would migrate toward the anode during electrophoresis.

A key element of the electrophoresis activity is the construction of gel boxes and the preparation of gels. In many laboratory courses, these parts of the process are invisible to students. Learning how the equipment works, students gain a deeper understanding of related concepts and processes, thus avoiding any "black box" misconceptions caused by technology overreliance [9]. Responses to surveys about the activity were significantly positive with highest scores for quality, organization, active participation, and enjoyment. Using relatively inexpensive and locally obtained materials, we offer a way to expose students to gel electrophoresis, a molecular technique central to modern biology research. Preliminary accounts of this work have been presented previously [10–12].

MATERIALS AND METHODS Gel Box and Power Supply

For agarose gel electrophoresis, a gel is poured and run horizontally, submerged in running buffer. Laboratory equipment has platinum wire electrodes positioned along the bottom of each end of the gel box, and the gel typically sits on a platform

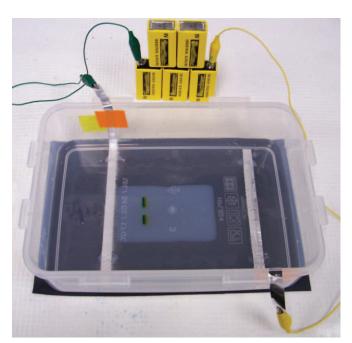


Fig. 1. Gel box constructed from a plastic container, aluminum foil electrodes, and 9-V batteries attached with wires and alligator clips.

separating two buffer chambers. One could construct a Plexiglas box that mimics commercially available systems, but household plastic containers work well. These should be scaled to fit the gel prepared and need to be wide enough that the gel does not touch the sides and long enough that the anode can be placed at least 2 cm from the end of the gel. No cover or shielding is needed, as the electrical current applied will be low and safe to touch. Gel boxes are simple constructions that should be made anew for each electrophoretic run.

There are several suitable materials for making electrodes. Platinum wire is expensive and difficult to obtain, while stainless steel wire also is not typically available locally. Bare copper wire (18 gauge), steel wire (baling wire or paper clips) can be used; however, these corrode quickly and often break during longer electrophoretic runs. Aluminum foil folded over several times to create an electrode strip $\sim\!\!1$ cm wide performed well (Fig. 1). Although there is significant corrosion and pitting of the aluminum foil electrodes during electrophoresis, the electrodes remain intact for normal runs. One must be careful to place the anode at least 2 cm away from the end of the gel as oxidation products can accumulate as white flocculent material near the anode, and these can cause the gel to erode or split during electrophoresis.

The power supply is typically the most expensive item required for electrophoresis in the research laboratory and introduces shock hazards. Fortunately, a group of 9-V batteries connected in series is suitable for electrophoresis. Five batteries yield 45 V with a low and safe current. The batteries can be used for two or three electrophoretic runs of 1 to 1.5 hr before being expended. In large classes with several groups constructing gel boxes, the batteries are likely the greatest cost of the activity. One has the option of using a transformer, such as a cell phone charger, after removing the connection plug and exposing the low-voltage output wires, but there is a greater shock hazard. Wires fitted with alligator clips are used to attach the batteries to the gel box electrodes.

Gel and Running Buffer

Horizontal submerged DNA gels are typically made of agarose, a purified form of agar composed of only neutral carbohydrate chains. One concern with using agar, rather than agarose, for gel electrophoresis is that charged carbohydrates in agar could cause bands to become diffuse due to electro-osmosis [13, 14]. This has not been a noticeable problem for the agars examined here. Agar is a vegetable (algal) gelatin powder, often called agar-agar, available in natural or Asian food stores and online. While Now brand agar (Now Foods, Bloomingdale, IL) or BactoAgar (Difco) are suitable, Telephone brand agar-agar (Sirian International, Bangkok, Thailand) performed best. Agaragar from Eden Foods (Clinton, MI) and Desi Mills (Toronto, CA) did not perform as well. Gels are formed with 1% or 2% agar (w/v), carefully melted in a microwave oven before casting.

Two different gel systems have been successful, with a minigel system giving the best results overall. Larger gels can be cast easily using a plastic container, and the gel can be cut to a rectangular shape (not required) before use. Thinner minigels run faster and stain more quickly. Although these can be poured on the surface of a smooth store gift card ($\sim 54 \times 85$ mm), with the surface tension of the gel solution retaining sufficient depth, it was easiest to pour the gel on the bottom of a clean glass baking dish. A rectangle, the size of a store gift card, is drawn as a heavy wide line of crayon or wax pencil, and the molten agar slowly poured to capacity.

Wells for loading DNA solutions are created in the gel using a comb. A good design has teeth along the long edge of a smooth store gift card created with a hole-puncher. Binder clips attached to either end allow the comb to be vertically positioned just above the surface of the casting tray. The wells should be as deep as possible, but several millimeters of gel

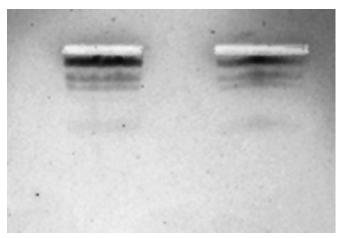


Fig. 2. DNA bands from an agar (Telephone; 2% w/v) gel with citrate buffer (Kraft Crystal Light lemonade; 8 g L $^{-1}$) run for 1 hr at 45 V and stained with methylene blue solution (Kordon; diluted 3000×). Several bands are visible from the lambda Hindlll ladder (Fisher; 10 μL ladder in 50% corn syrup solution) applied to both lanes.

must remain below the teeth of the comb to form the bottoms of the wells. The cooled gels can be lifted out of the tray with a spatula; adding some running buffer to the tray helps to dislodge a gel that is sticking.

Agarose gel electrophoresis systems use the same buffer for both casting and running the gel. It is critical to buffer the pH so that DNA is not degraded by high or low pH and an electrolyte (salt) is needed to carry the current. Research laboratories use Tris as the pH buffer (pH 7 to 8) and as an electrolyte, in Tris-acetic acid-EDTA (TAE), Tris-borate-EDTA (TBE), or Tris-glycine buffer systems. Unfortunately, Tris is not a household product. A bicarbonate buffer system has been reported [5], and electrophoresis is possible using 2 g L^{-1} baking soda (sodium bicarbonate) with 0.05 g L⁻¹ NaCl as the running buffer. One of the best running buffers was the citric acid and sodium citrate formulations found in common dry beverage mixes. Tang Breakfast Drink (Kraft Foods, Rye Brook, NY; 8 g L⁻¹) was suitable, but it is highly colored. Crystal Light lemonade (Kraft; 8 g L⁻¹) was an excellent running buffer and allowed for good staining of the DNA bands. Note that these mixes are typically acidic, and the pH needs to be set to near 7 (pH paper is available for aquaria) before use to protect DNA. Buffers are available for aquaria and these are already set to pH 7, making their use even easier. The best buffer system was an aquarium Neutral pH Regulator (Seachem Laboratories, Covington, GA) containing a mixture of potassium phosphates. Magnesium citrate solution (Kroger, Cincinnati, OH; 1.75 g fl. oz. diluted seven-fold) and Alka-Seltzer (Bayer; 8 g L⁻¹) did not perform well and gels cast with sodium borate buffer (20 Mule Team Borax, Dial Corp., Scottsdale, AZ; 8 g L⁻¹) were mottled, showing bright spots on staining.

The progress of electrophoresis is typically followed by watching dye molecules migrate through the gel. Food coloring dyes are suitable and can be added to DNA preparations. To make the applied samples sink into the wells, it is helpful to add dense glycerol (glycerin) or corn syrup to the extracts. The viscosity of the sample can be adjusted for best performance with the loading method chosen (syringe, pipetter, dropper, or toothpick).

Staining

After electrophoresis, DNA bands are visualized in gels by staining. In the research laboratory, stains that intercalate between the DNA bases are used, such as ethidium bromide or

Syto13. These stains are too hazardous for the classroom due to their high mutagenicity and teratogenicity. Methylene blue (Kordon, Hayward, CA) is a safe alternative, available from pet supply stores as a 2.3% solution, that is diluted 3000× or more with water for staining (Fig. 2). Crystal violet, marketed in pharmacies as a 2% solution called gentian violet (Humco, Texarkana, TX), stained DNA somewhat better than methylene blue. Malachite green (Rid-Ich+, Kordon; 125× dilution) did not work as well. A variety of RIT clothing dyes (Phoenix Brand, Stamford, CT; 0.25 g L-1) was tested, but none stained DNA very well. Some staining was observed with navy blue, royal blue, dark green, purple, and black. Mrs. Stewart's Liquid Bluing (Bloomington, MN), a suspension of Prussian Blue, was not effective. Stained DNA bands were visualized with white light, either on a light box (light source behind opaque diffusing plastic) or after placing the gel in a clear plastic bag and holding it up to a bright window.

Safety and Disposal

The mainly edible materials used here pose few health hazards. Care should be taken when working with hot agar solutions, donning appropriate thermal gloves or kitchen mitts. Agar solutions tend to boil over or superheat and bump, so be careful. A series of short bursts (10 sec) in the microwave is best and be wary of swirling hot liquids. It is recommended that students wear gloves and eye protection when working with stains. All the materials can be sinked or disposed of as nonhazardous trash, with the exception of expended batteries, which require special disposal.

Analysis

After staining, bands of DNA molecules should be visible in the gel. The distance that DNA molecules migrate during electrophoresis is dependent on the size of the molecule, the composition of the buffer, the length of electrophoresis, the agar content of the gel, and the amount of current applied. Each gel will run somewhat differently and like chromatographic procedures, the distance migrated by a DNA band is usually reported relative to the migration of a molecule of known size. DNA length markers, called ladders, are available commercially but can be costly. It is possible to create suitable markers after shearing genomic DNA by several rapid passages through a syringe needle, electrophoresing the sheared DNA, and extracting (by soaking in buffer) sections of the gel at different points along the smear. The migration of the DNA bands can also be reported relative to the migration of one of the running dyes. Migration is measured either directly on the gel or by capturing an image that includes a ruler. Distance is measured from the bottom of the well to the middle of the band. If a ladder is used, a standard curve can be generated from the distance migrated and the known lengths of the DNA fragments. Teachers also can supply students with a table of migration distances and molecular lengths or assign values to the running dyes. Students should plot the standard curve and then determine the sizes of their unknown DNA fragments either directly from the graph or by generating an equation that describes the line. If several groups are running the same samples, results can be pooled and simple statistical analyses (standard deviation and Student t-test) applied to determine the variance of the data.

RESULTS Recommended Procedure

The gel box is made from a plastic storage container (14 \times 20 \times 5 cm, 1.6 L) and fitted with aluminum foil electrodes (1 \times 25 cm; four-layer folded strips) set 14 cm apart, attached with adhesive tape or glue. The elec-

TABLE I
Activity assessment survey

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Question	Score ± SD ^a
The gel laboratory sessions were well organized	4.3 ± 0.8
2. The experimental steps were explained well	4.2 ± 0.8
The gel laboratory stimulated active participation	4.1 ± 0.9
4. I was motivated to see the results	4.1 ± 0.9
The experiment worked well for my group	4.1 ± 0.9
I was unhappy that we used low-cost materials	2.1 ± 1.0
7. The gel laboratory made me think creatively	3.7 ± 0.8
8. I felt like I was doing real research	3.5 ± 1.0
Using new laboratory equipment raises my interest in science	4.0 ± 0.9
 The gel laboratory included up-to-date information 	4.0 ± 0.9
11. My teacher could answer my questions	4.1 ± 1.1
 The gel laboratory helped me understand important concepts 	3.9 ± 0.9
 My understanding of DNA is better after the gel laboratory 	3.7 ± 1.1
14. I learned how to use micropipetters	4.0 ± 1.2
 I feel like I learned something from this activity 	4.1 ± 0.8
16. The gel laboratory was relevant to my class	4.2 ± 0.8
17. The gel laboratory was a valuable learning tool	3.9 ± 1.0
 The activity made me want to do more experiments 	3.9 ± 1.0
19. The gel laboratory was of high quality	4.0 ± 0.8
20. It was fun to do the gel laboratory	4.3 ± 0.8

^a Likert-type scale: 1 = strongly disagree; 2 = disagree; 3 = neither agree nor disagree; 4 = agree; 5 = strongly agree.

trodes are connected to five 9-V batteries in series using wires fitted with alligator clips. Gels were cast in a clean glass baking dish (9 \times 13 in), where a rectangle (8.5 \times 5.5 cm) was drawn with a wax crayon. Telephone brand agar (1 g) was melted using a microwave oven in 50 mL of running buffer (3 g L⁻¹ Seachem Neutral pH Regulator). After carefully pouring the molten agar into the crayon rectangle until full, a comb made from a plastic store gift card and binder clips was used to form wells about 1 cm from the end of the gel. When the gel cooled (10-20 min), the comb was removed, the gel lifted from the casting tray, placed in the gel box with the wells close to the cathode, and submerged (to 1 cm above gel) in running buffer. DNA samples (\sim 10 μ L) such as a standard lambda HindIII ladder (Fisher Scientific; ~\$1 per lane) or genomic extract were added 1:1 in loading dye (one drop corn syrup with three drops green food coloring) using a pipetter. Electrophoresis proceeded for 1 to 1.5 hr, while observing the migration of the tracking dyes. Gels were stained submerged in a gentian violet (10,000× dilution) bath for 12 to 72 hr without shaking at room temperature. DNA bands were visualized with backlighting and photographed for further analysis.

Typical Results

The result of a typical gel separating a lambda HindIII ladder is shown in Fig. 2. Notice that discrete bands are visible after staining, each representing a differently sized DNA fragment of the ladder. Students in our classroom

test were successful in visualizing DNA bands in their gels. Genomic DNA tends to form diffuse, high-molecular weight bands but also can produce more than one band. These may be of different sizes or they may be different conformations (e.g. supercoiled) of the genomic DNA that lead to changes in electrophoretic mobility. Electrophoresing for longer periods will move the DNA bands further into the gel and provide better separation. A wider range of band sizes can be obtained by first shearing genomic DNA by repetitive rapid passage through a syringe needle or by using commercially available restriction enzymes.

Scheduling and Assessment

Depending on bell schedules, it might be best to separate steps in the protocol into different blocks. In our trials, the first block was used for an activity to practice pipetting and discussions about the procedure and its rationale. The gel boxes were constructed, gels poured, loaded, and run in the second block (using a lambda HindIII ladder). The teacher removed the gels from stain later on and stored these for a third block, where the band patterns were documented and analyzed. Students were given a worksheet to complete while waiting for procedures to complete that included questions about the basis for electrophoresis, the negatively charged backbone DNA that allows for electrophoretic separation. and the determination of molecular lengths by analysis of the banding patterns. Open-ended questions, studentcentered responses, appropriate wait-time and nonverbal behaviors were used to engage dialogue and draw out ideas [15-18]. Students generated a standard laboratory report and were graded according to their continual interactions throughout the lessons. Teachers also can assess students' understanding through extension projects (see below).

Student Surveys

The test class at NEM was mainly sophomores in five groups of 4–5 students each. Surveys using a Likert-type scale were completed by all 21 students, with appropriate parental and school permission (Table I). Overall, scores were 4.0 or above, indicating that students appreciated the activity and found it valuable. The scores were significantly (p < 0.05) higher than expected for every question (except Q13) using chi-square analysis and the expectation of an even distribution across the responses. Students saw the activity as connected to their coursework and up-to-date. The students felt that they actively participated, learned something valuable, and wanted to do more science investigations. The highest scores were in agreement that the activity was fun and well organized. The students were not disappointed that the activity used low-cost materials rather than laboratory equipment. Although the average responses were positive, two of the students had lower scores overall, falling just below neutral (3.0). Taken together, these survey results were significantly higher (p < 0.01) than expected and suggest that students viewed the electrophoresis activity as successful and a valuable addition to an introductory high school biology course. Content surveys previously conducted after similar electrophoretic activities using laboratory equipment at NEM and other WPS high schools showed a significant increase in content knowledge specific to the activity [11]. It must be noted that a single classroom activity is not expected, by itself, to appreciably increase performance on broad-based science assessments or overall course grades.

DISCUSSION From Pricey to Priceless Learning

A safe inexpensive electrophoresis activity for use in high school biology classrooms or homeschool settings is presented that addresses several points in Life Science Standard C, including aspects of cell biology, genetics, and evolution. The activity also broadly addresses all the points in Standard A, Science as Inquiry, as well as Standard E, Science and Technology. It relies on technology to observe phenomena that have scientific explanations. Students can then use mathematics to analyze and communicate their results. Furthermore, by starting with the construction of the apparatus itself, the activity more closely mimics research, where commercial equipment and reagent kits may not be available for specific laboratory protocols. The simple low-cost system described here can be incorporated into general biology courses in sections on genetics or diversity to give secondary students a hands-on experience generally reserved for college laboratory courses. This can be done at a much lower cost and with greater safety than with standard protocols. Using our scheme, more teachers should be able to expose their students to central techniques in molecular biology that are critical for life sciences research today.

Extending the Activities

Chemical and physical treatments of DNA extracts can change the banding pattern observed after electrophoresis. Genomic DNA tends to appear as a diffuse band of high-molecular weight. If the DNA is sheared by rapid passage through a syringe needle, the fragments form a smear in the lane. The smear will appear at lower molecular weights when the DNA extract is treated with a greater number of forceful passages through the syringe. Chemicals such as acid and bases can affect DNA stability and fragment or degrade DNA. Students or teachers can generate DNA ladders by cutting blocks out of DNA smears and soaking the DNA out of the gel fragments with buffer. This would allow for standard curves to be made to obtain better relative front values. DNA ladders also are available commercially.

The electrophoresis activity could be built into a forensic investigation, where DNA patterns are compared between "crime scene" and "suspect" DNA extracts. This works best when several restriction enzymes are used such that the enzymes are mock "DNA extracts" and a single DNA extract (lambda phage DNA; Fisher Scientific; typically \$1 per lane) is used as the mock "enzymes." Relatively inexpensive restriction enzymes

such as EcoR1 or BamHI (Fisher Scientific; typically < \$1 per reaction) can be used at room temperature to cut DNA during overnight incubations.

A more extensive option begins with a microbiology system developed using household materials that can safely and inexpensively maintain salt-tolerant bacterial isolates for study [19]. Genomic DNA extracts (several rapid freeze-thaw cycles using a dry ice-ethanol bath and hot water) from the isolates can be electrophoresed and the genomic band excised and soaked in water to release the DNA. The students can use the extracts as part of a mock PCR activity. Teachers create mock PCR reagents (nucleotides, polymerase enzyme, buffer, and primers) with tubes of colored water and students assemble the reaction mixture with their DNA extract. The reactions would then be "sent off" for mock PCR amplification and base sequencing "at a local university." The students would be given the results, a file containing the DNA sequence for a bacterial 16S rRNA gene obtained from GenBank. Tools on the Ribosomal Database Project website [20] can be used for analysis of the sequence and the creation of phylogenetic trees. This can be coupled to the generation of phylogenetic trees that include humans and related animals, again targeting the relationship between species identity and genetic content.

Use In Other Science Courses

The electrophoresis activity was developed for high school biology classrooms but can be used in middle school classrooms. The activity addresses middle school Life Science Standard C. This includes standards within Reproduction and Heredity (#3–5), Diversity and Adaptation of Organisms (#1–2), and Structure and Function of Living Systems (#1–2). Keys points involve the chemical nature of DNA and that an organism's genetic makeup determines its traits and identity.

The activity can be used to address high school Physical Science Standard B, specifically within Structure and Properties of Matter (#4) and Motion and Forces (#1, 3, 4). The latter standard includes an understanding that charged particles move in electric fields and that molecules move when a force is applied. In a physical science course, electrophoresis of dyes rather than DNA would be appropriate. Many fabric and food dyes will separate into several differently colored bands when electrophoresed. Some dyes may be positively charged and move toward the cathode, therefore, casting wells in the middle of the gel may be useful. The rate and extent of movement of dye bands can be measured and mathematical analysis can include relative front values, where the migration of bands is expressed as a fraction of a standard fast-moving dye band. Gels will run somewhat differently for each laboratory group, and band migration data from the entire class (or school or district) can be pooled and analyzed using simple statistics (standard deviation and Student t-test).

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