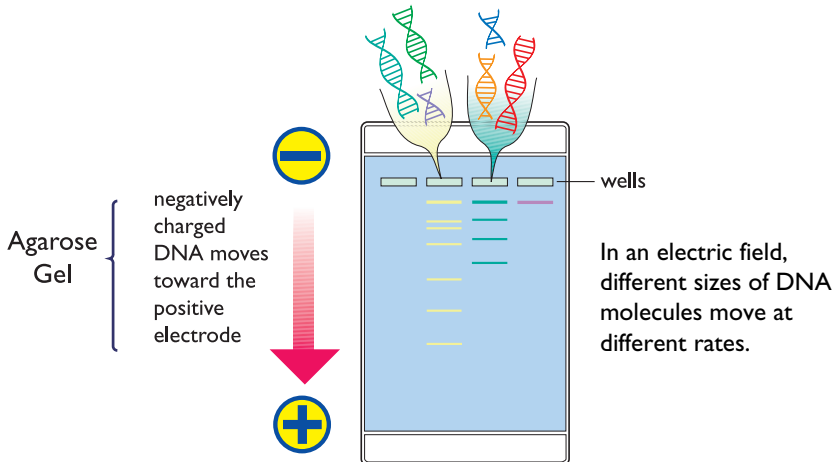


21-1012, 21-1014

Exploring Electrophoresis and Forensics

Teacher's Manual



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Contacting Carolina Biological

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Acknowledgments

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Background

This exercise is technically simple. Students cast agarose gels, load pre-cut DNA into the gels, perform electrophoresis, and interpret the banding patterns to determine if one of the suspects' DNA profiles matches crime scene evidence. The science behind the activity, however, is not simple. In the student guide, students are given a brief explanation of the science. Below is a more detailed explanation of how PCR and DNA fingerprinting work.

The Polymerase Chain Reaction

The polymerase chain reaction (PCR) is used to make many copies of a defined segment of a DNA molecule. Here's how it works. First, you decide what DNA segment you wish to duplicate, or amplify. Then you obtain two short single-stranded DNA molecules that are complementary to the very ends of the segment. These two short molecules must have specific characteristics. Each of the single-stranded molecules must have a base sequence that is complementary to only one strand of the target DNA, and each one must be complementary to only one end of the segment. Furthermore, if you imagine these short molecules base paired to the complementary regions in the target DNA molecule, their 3' ends would point toward each other. These short, single-stranded molecules are the **primers** for PCR.

To begin the chain reaction, a large number of primers is mixed with the target molecule in a test tube containing DNA polymerase enzyme, buffer, and many nucleotides. Nucleotides are the building blocks of DNA. This mixture is heated to almost boiling, so that the base pairs holding the two strands of the parental molecule separate, or denature.

Next, the mixture is allowed to cool. Ordinarily, the two strands of the target DNA region would eventually line up and re-form their base pairs. However, there are so many molecules of primers in the mixture that the short primers find their complementary sites on the target strands before the two target strands can line up correctly for base pairing. Therefore a primer molecule base pairs, or hybridizes, to each of the target strands.

Now DNA polymerase enzyme adds nucleotides to the 3' end of each primer, using the bases on the target strand as a template. New complementary strands are made, and the 5' end of each is formed by a primer. In this manner, two double-stranded DNA fragments are formed where before there was only one.

This process of denaturation, hybridization, and DNA synthesis is repeated over and over. Each time, the number of DNA fragments in the mixture is doubled. When this process is repeated 30 times, which is a typical number of PCR cycles, 2^{30} times as much DNA as was in the original region of DNA is the result. This is more than a billion new fragments of DNA from the region between the two primers.

PCR and DNA Fingerprinting

How is PCR used to generate a DNA fingerprint? First, let's remember the basic problem in human DNA fingerprinting. Humans have about 3 billion base pairs of DNA, most of which are identical from one person to another. This is too much DNA to examine simply by cutting with restriction enzymes and comparing banding patterns in a gel. Those 3 billion base pairs generate too many fragments, producing an overlapping smear of bands in a gel. Fortunately, many of the differences in the DNA of one person and another are concentrated at certain places along chromosomes. To generate a DNA fingerprint, scientists have found ways to look specifically at these regions where differences are concentrated. One of the ways they look is through PCR.

PCR is used to detect a specific type of difference for DNA fingerprinting. Human DNA contains end-to-end, or tandem, repeats of different short DNA sequences (from as few as 3 bases up to 30 or more bases long) at many places scattered throughout the chromosomes. Although the chromosomal locations and the base sequences of the repeats at a given site are the same from person to person, the *number* of the repeats at a given location varies highly from individual to individual. This means that the DNA fragments that result from a PCR reaction are different sizes, because the area of DNA being amplified has a different number of repeats. Since an individual may have different numbers of repeats on the maternal and paternal members of a chromosome pair, we may obtain one or two DNA fragments of different sizes from a PCR reaction using a single primer pair.

In this simplified activity, we are only looking at the results of PCR from one region in the DNA. In actuality, you would need to look at the results of PCR from multiple regions in the chromosome to definitively identify an individual. There might be a 1 in 20 chance that two individuals would randomly match from one particular location to another. There might also be a 1 in 20 chance that two individuals would match at a second location in the DNA. The chance of those two individuals matching at both locations would be 1 in 20×20 , or 1 in 400. Increasing the number of locations in an individual's DNA that are examined

increases the probability that you can identify an individual based on their DNA.

Materials

	<i>included in teacher demo kit</i>	<i>included in classroom kit</i>
	(21-1012)	(21-1014)
Suspect 1 DNA	2 vials	10 vials
Suspect 2 DNA	2 vials	10 vials
Victim DNA	2 vials	10 vials
Evidence DNA (DNA samples sent in foil pouch)	2 vials	10 vials
Agarose, 0.8% melt-n-pour	30 mL	1.2 grams
TBE buffer, 20×	50 mL	50 mL
<i>Carolina BLU™</i> final stain concentrate	50 mL	50 mL
Gel box	1	5
Comb	1	5
Electrode set	1	5
Syringes, 1 cc	1	5
Tubing	(with syringe)	10 cm
Yellow tips	6	30
Carbon fiber sheet (11 cm × 10 cm)	1	3
Teacher's Manual	1	1
Student's Guide	1	5

The classroom set includes materials for 5 groups of students to perform the lab activity twice. The demonstration set includes materials for two demonstrations.

Preparation of Materials

Agarose

For demo kit

The agarose can be melted by microwaving it briefly. When it is dissolved, no lumps or fibers should be visible.

For classroom kit

Make 0.8% agarose gel by dissolving 1.2 g of agarose powder in 150 mL of 1× TBE solution. This provides enough agarose for approximately 15 gels (each gel box will hold 10–12 mL agarose).

Note: Do not dissolve the agarose in water. The easiest way to dissolve the agarose is to use a microwave oven. It is important that the concentration of agarose be correct. Don't be tempted to prepare a small volume. Make up at least 150 mL, use what you need, and keep the rest in an airtight container to prevent desiccation.

Electrophoresis (TBE) Buffer Solution

Add 25 mL of 20× TBE to 475 mL distilled or deionized water.

Note: The buffer solution may be reused several times.

Syringe pipettors

For classroom kit

The adapter tubing should be cut into pieces approximately 5–7mm long. These pieces of adapter tubing should be placed on the tips of the syringes. They will ensure a tight fit of the yellow tips.

Carbon Fiber Electrode Tissue

May be cut by students, as described in the Student's Guide.

Materials needed but not included:

Distilled water

9-V batteries

Batteries

The length of time required for electrophoresis varies with the number of batteries used. For overnight electrophoresis, 1 battery is appropriate. For quickest results (40 min), 5 batteries are appropriate. The 5 batteries are used in a cluster, made by snapping the positive terminal on one battery to the negative terminal on the next battery, and so on.

Laboratory Safety

The manufacturers of this product have made every effort to check that recognized hazards have been identified and that suitable precautions are suggested. Where possible, the proposed procedures are in accordance with commonly adopted general risk assessments. If a

special risk assessment may be necessary, this has been indicated. However, users should be aware that errors and omissions can be made, and that different employers and educational authorities adopt different standards. Therefore, before initiating any activity, users should always carry out their own risk assessment. In particular, any local rules issued by employers or educational authorities **MUST** be obeyed, regardless of whatever else is suggested by the manufacturers.

Uncut and Cut DNA

The DNA provided with this kit is quite safe to use. Living organisms are not used, so there is no need to follow strict aseptic techniques. However, cleanliness is important to prevent cross-contamination and to ensure success. Spilled DNA solution should be wiped up promptly.

Agarose Gel

If a microwave oven is used to melt the agarose gel, ensure that the gel is placed in an unsealed container. If a microwave oven is not available, a boiling water bath or hotplate may be used instead. On a hotplate, the gel must be swirled as it melts to prevent charring. The use of a bunsen burner to melt agarose is not recommended.

Warning! Hot, molten agarose can scald, so it must be handled with care. Let the molten agarose cool until it is comfortable to handle before pouring the gel.

Electrode Tissue (Carbon fiber)

The carbon fiber electrode material may release small fibers, which can cause minor skin irritation if you handle the tissue a lot. It is a wise precaution to wear protective gloves if you find the tissue unpleasant to handle. However, the fibers released are too large to enter the lungs, so it is not necessary to wear a face mask. In addition, the fibers are soluble in body fluids and are completely biodegradable.

Electrical Supply

Warning! The gel electrophoresis equipment was designed to be used at low voltages (≤ 45 volts) with dry cell batteries. Under no circumstances should this voltage be exceeded, as the live electrical components are not isolated from the user.

Serious or lethal electrical shock could result if the equipment is connected directly to a power supply.

Note: Good results will be achieved if the gel is run between 9 and 35 volts. At higher voltages, the DNA fragments migrate faster, but they will not separate as well.

Carolina BLU™

When used as directed, *Carolina BLU™* presents no serious safety hazard, although care should be taken to prevent splashes on the skin or eyes (e.g., wear protective gloves and safety glasses when handling the stain). Used stain can be diluted with water and washed down the drain.

TBE Buffer

(Tris-Borate-EDTA)

When diluted and used as directed, this buffer presents no serious safety hazards. Used buffer can be washed down the drain.

Reuse of Materials

Electrophoresis Buffer Solution (TBE)

The TBE buffer solution can be retained and reused several times if desired. Eventually it becomes “tired” due to electrolysis—but at the low voltages encountered with this equipment this should not be evident for some time.

Carolina BLU™

The *Carolina BLU™* stain may also be reused. After many uses its effectiveness will be reduced as it is gradually diluted with buffer solution washed from the gel. With elderly stain you may find it necessary to soak the gel for longer than the 4 minutes that is suggested in the student’s booklet.

Agarose

Agarose is very expensive. Damaged, unused gel, e.g., from a poorly cast gel, may be remelted and used again. However, do not attempt to reuse agarose that you’ve already used for electrophoresis. Agarose is not like nutrient agar— nothing will grow on it once it’s been made up, so you can prepare a large batch and just use what you need. It can be remelted and allowed to set many times (boiling is required to melt the agarose; once molten it can be maintained in that state at 55–60°C). Prepared agarose gel should be stored in an airtight container to prevent desiccation.

Syringe Tips and Tubes

Both the syringe tips and tubes are autoclavable and may be washed, sterilized, and reused if you wish.

Storage of Reagents

Uncut and Cut DNA

The DNA in this kit has been stabilized for storage at room temperature (20°C). Do not store it in direct sunlight. During prolonged storage, water may evaporate from the DNA solution. Although this does not damage the DNA, it reduces the volume of the solution and increases its viscosity. We therefore recommend that the DNA be refrigerated (at about 4°C) if it is to be stored for more than 6 weeks. If your samples become viscous, add 10–15 μL of distilled water to each one immediately before use.

Buffer Solutions

Buffer concentrate should be stored at room temperature. Diluted buffer solution should be stored in a refrigerator at about 4°C.

Sometimes crystals of EDTA come out of solution in the concentrated TBE buffer. It is important that these are redissolved before you dilute the buffer. Do this by heating the solution, e.g., by standing the bottle in a heated water bath.

Carolina BLU™

The stain may be stored at room temperature.

Hints and Tips

Using Syringes

Students' ability to dispense precise volumes using the syringes will improve with experience. It is therefore a good idea to encourage them to practice transferring liquids (e.g., food coloring) before starting work with expensive DNA. Some extra syringe tips are provided in the kit for this purpose. To give students practice, gels may be cast from cheap agar (use a 1% solution) rather than agarose. When practicing, use water rather than buffer over the gel.

Note: For your convenience, you may also purchase Practice Pipetting Stations from Carolina Biological Supply Company (catalog # 21-1145).

DNA Solution

Should the DNA solution become scattered inside the tube, tap the tube repeatedly on a hard surface to return the liquid to the bottom of the tube. A centrifuge may also be used to spin the DNA down inside the tube.

Melting Agarose

Ensure that the container used to prepare the agarose gel is clean. Tiny flecks of dust will not affect the way the gel runs, but they can prove a nuisance when you are trying to see faint bands in the gel.

For convenience, dissolve and melt the agarose using a microwave oven. Less than a minute at full power in a 940-W oven is sufficient for 100 mL of gel. The container (flask or beaker) used to hold the molten agarose must not be sealed, but covered lightly with plastic film that has been punctured with one of two small holes. Halfway through the heating cycle, swirl the gel to ensure that it is thoroughly mixed.

Agarose gel can be prepared using a hotplate. If this is done, the gel must be swirled as it melts, to prevent charring. Better still, stand the container of agarose in a saucepan of boiling water. The use of a Bunsen burner to melt agarose is not recommended.

Once melted, the gel may be kept in a molten state by standing the container in a water bath at 60°C until the gel is needed. Take care when handling the molten gel; it will be very hot and can scald. The gel should be allowed to cool before it is poured.

Batteries

Dry cells (the alkaline or zinc-carbon types) are suitable for use with this equipment. Several "runs" may be obtained from one battery. Rechargeable nickel-cadmium cells also give very good results, but must be completely discharged and then recharged after one or two runs to maintain their performance.

Gel Takes Too Long to Run

With 2 new or freshly charged 9-V batteries the loading dye should move about 5 mm per hour. At a lower voltage the loading dye will move more slowly.

If, after the first 20 minutes, the loading dye does not seem to have moved and bubbles are not visible at the cathode, check the electrical contacts between the batteries and the electrodes. Ensure that there is enough buffer above the gel to cover the plastic ridges at each end of the tank, but not so much that most of the current passes through the

buffer solution rather than the gel. Remember that during long runs (e.g., overnight) or in a warm environment, liquid may evaporate from the buffer. The tank should be covered loosely (e.g., with a plastic bag) to reduce such evaporation.

Corrosion of Alligator Clips

The alligator clip used at the anode will slowly corrode due to electrolysis. This can discolor the buffer solution and gel, but will not interfere with the electrophoresis. Eventually, you will need to replace the clip.

Viewing the Stained Gel

The bands are seen most easily by holding the gel at an angle against a well-lit surface. Several authors suggest that a yellow filter improves the contrast between stained bands and the background. While this may be true if you are taking photographs, filters can make it more difficult to spot very faint bands with the naked eye. A magnifying lens can be useful; flat fresnel lenses the size of a credit card (sold for use with atlases) are ideal. If the contrast between the background and the DNA bands is strong, the gel may be viewed using an overhead projector.

Further Resources

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- Miller, M. B. 1993. DNA technology in schools: a straightforward approach. *BIOtechnology Education* 4 (1): 15–21.
- Miller, M. B., and G. A. Russell. 1996. Practical DNA technology in school—2: Computer analysis of bacteriophage lambda base sequence. *Journal of Biological Education* 29 (3): 176–183.
- Mullis, K., 1990. The unusual origin of the polymerase chain reaction. *Scientific American* 262 (4): 56–65.

Nowak, R., 1994. Forensic DNA goes to court with O. J. *Science* 265:
1352–1354.

The Polymerase Chain Reaction. Video. Explains PCR through the use of
simple models. Carolina catalog #21-2734.

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