Background Information

PRINCIPLES OF GEL ELECTROPHORESIS

Gel electrophoresis is widely used to separate molecules based upon charge, size and shape. It is particularly useful in separating charged biomolecules such as DNA, RNA and proteins. This technique possesses great resolving power, yet is relatively simple and straightforward to perform.

Agarose, a polysaccharide derived from seaweed, is commonly used to form the separation matrix used for gel electrophoresis. To make a gel, solid agarose powder is added to buffer and melted by boiling. The buffer controls the pH of the solution throughout the electrophoresis process, which is important to the charge and stability of biological molecules. Once the solution has cooled to approximately 60° C, it is poured into a gel tray to solidify. A special comb is used to form depressions in the gel called loading wells.

Once solidified, the gel is placed in a horizontal electrophoresis chamber and covered with a pH-balanced buffer. Electrodes placed at each end of the electrophoresis chamber generate current when connected to a direct current power supply. The buffer contains ions necessary to conduct the electrical current.

Samples are prepared for electrophoresis by mixing them with glycerol or sucrose, which makes them denser than the electrophoresis buffer. When the samples are loaded into the wells, the dense samples sink through the buffer and remain in the wells. An electrical current is passed through the gel to drive molecules through the gel. Generally, the higher the applied voltage, the faster the samples are separated by electrophoresis. Once the current is applied, the biomolecules in the sample are pulled into the gel matrix. At first glance, an agarose gel appears to be a solid at room temperature. On the molecular level, the gel contains small channels through which the molecules can pass. These pores act as a molecular sieve that affects the rate at which a molecule can migrate through the gel.

Factors such as the molecular charge, size and shape, together with buffer conditions, gel concentrations and voltage, can affect the mobility of molecules in a gel. For example, small DNA fragments move through these holes easily, but large DNA fragments have a more difficult time squeezing through the tunnels. Because molecules with dissimilar sizes travel at different speeds, they become separated and form discrete "bands" within the gel. Given two molecules of the same molecular weight and shape, like dyes, the one with the greater amount of charge will migrate faster. Molecules with a net negative charge migrate towards the positive electrode (anode) while net positively charged molecules migrate towards the negative electrode (cathode). Furthermore, molecules may have the same molecular weight and charge but different shapes. Molecules having a more compact shape, like a sphere, would move through the pores more quickly than molecules with a looser conformation.

THE POLYMERASE CHAIN REACTION (PCR)

In 1984, Dr. Kary Mullis revolutionized the field of molecular biology when he devised a simple and elegant method to copy specific pieces of DNA. Recognizing that an initial step in DNA replication in a cell's nucleus is the binding of RNA primers, Mullis discovered that he could replicate DNA in vitro using short, synthetic DNA primers and DNA polymerase I. Furthermore, because researchers can specify a primer's sequence to target a specific gene, this method allowed for the rapid amplification of a selected DNA sequence. For the development of this technique, known today as the Polymerase Chain Reaction (or PCR), Mullis was awarded the Nobel Prize in Chemistry in 1993.

In order to amplify DNA, purified double-stranded DNA is mixed with the short DNA primers, a thermostable DNA polymerase (*Taq*) and nucleotides. The mixture is heated to 94°C to "denature" (i.e., unzip into single strands) the DNA duplex. Next, the sample is cooled to 45°C-60°C, allowing the primers to base pair with their target DNA sequences (a step known as "annealing"). Lastly, the temperature is raised again, to 72°C, the optimal temperature at which *Taq* polymerase will extend the primer to synthesize a new strand of DNA. Each cycle (denaturation, annealing, extension) doubles the amount of target DNA. Today, a specialized machine, called a "thermal cycler" or "PCR machine", is used to rapidly heat and cool the samples. As a result, a PCR cycle can be completed in less than 5 minutes; 20-40 cycles produce sufficient DNA for analysis.

Background Information

Because of its ease of use and its ability to rapidly amplify DNA, PCR has become indispensible in medical and life sciences labs, replacing the time-intensive Southern blot as the method of choice. For example, today's research laboratories can quickly create copies of a specific region of DNA for cloning applications. Medical diagnostics use PCR to identify genetic mutations and infectious agents. In addition, because amplification by PCR requires very little starting material, it is ideal for forensic analysis of biological samples or determination of paternity.

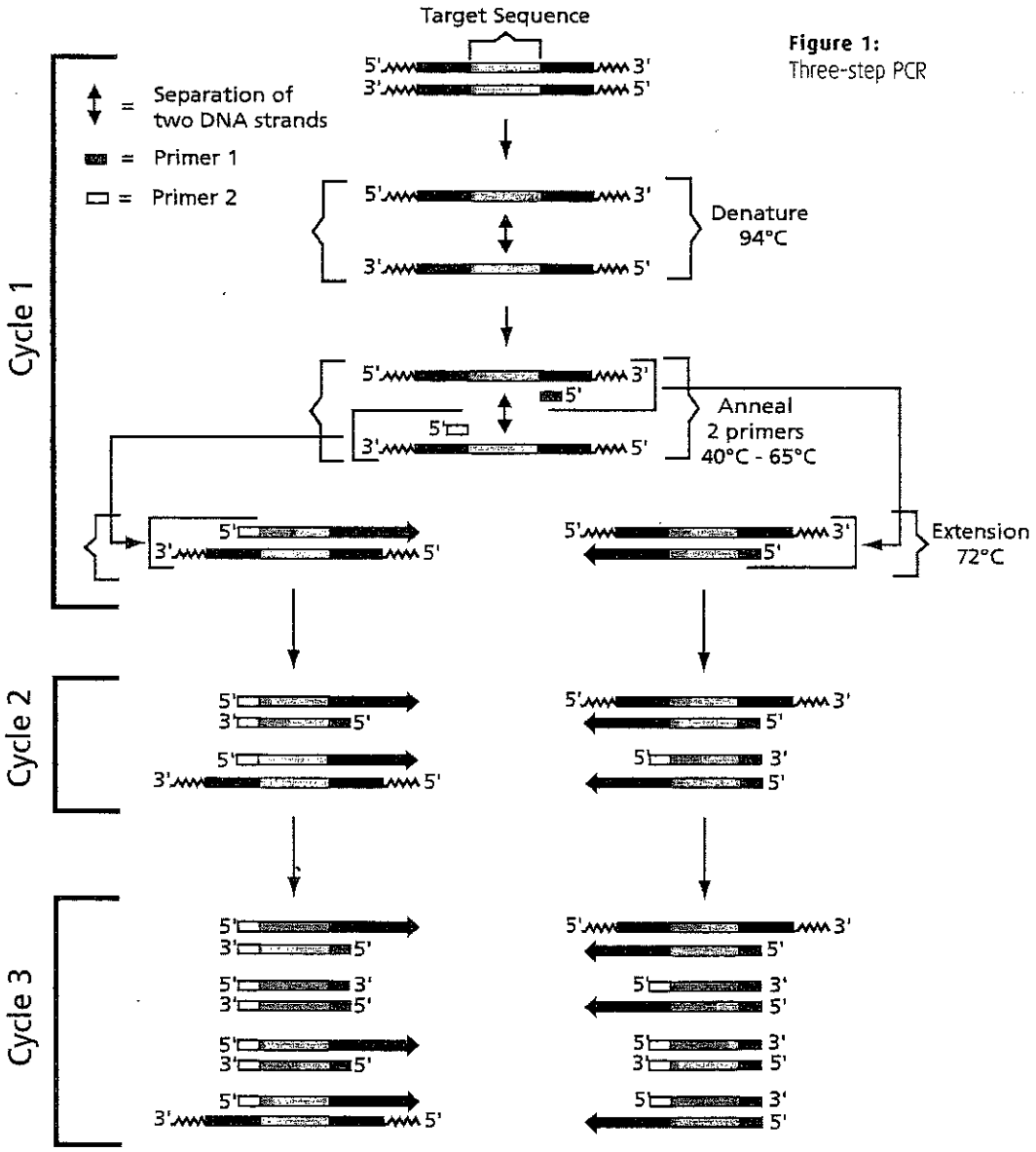


Figure 1: Three-step PCR

Background Information

LESSON PLAN OPTION A:
USING DYES TO SIMULATE A CRIME SCENE/DNA FINGERPRINTING ACTIVITY

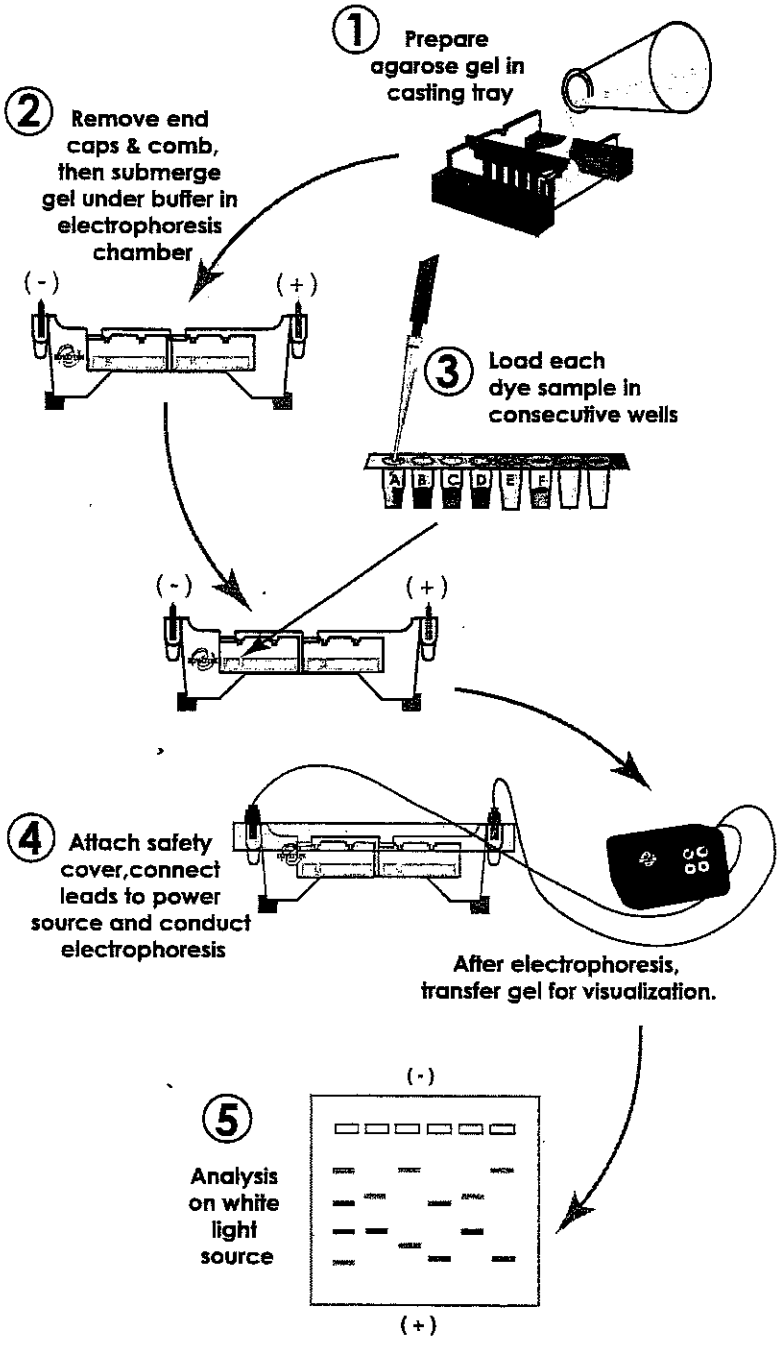
In this scenario, students will analyze PCR reactions obtained from different suspects and compare them to a crime scene sample.

A Standard Dye Marker
B Crime scene PCR reaction
C PCR control reaction
D Suspect 1 PCR reaction
E Suspect 2 PCR reaction
F Suspect 3 PCR reaction

In humans, DNA is packaged into 23 pairs of chromosomes that are inherited from an individual's biological parents. Although most of this genetic material is identical in every person, small differences, or "polymorphisms", in the DNA sequence occur throughout the genome. For example, the simplest difference is a Single Nucleotide Polymorphism (or SNP). Changes in the number and location of restriction enzyme sites result in Restriction Fragment Length Polymorphisms (or RFLPs). Short repetitive stretches of DNA at specific locations in the genome can vary in number to produce STRs (Short Tandem Repeats) and VNTRs (Variable Number of Tandem Repeats). Although most polymorphisms occur in non-coding regions of DNA, those that disrupt a gene can result in disease. Medical diagnostic tests can identify specific polymorphisms associated with disease.

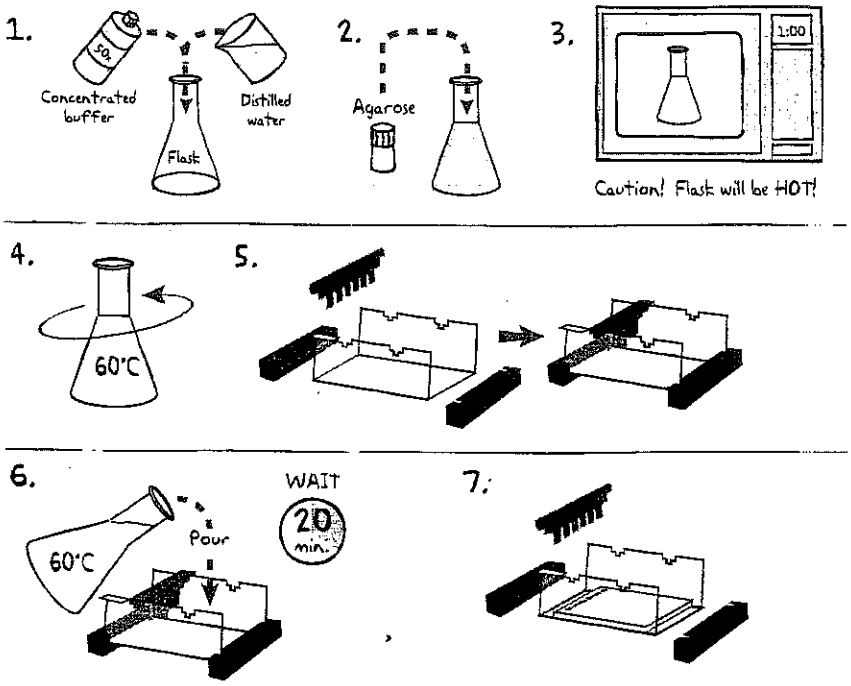
Analyzing several different polymorphisms within a person's genome generates a unique DNA "fingerprint". DNA fingerprints can allow us to distinguish one individual from another. Because polymorphisms are inherited, DNA fingerprints can also be used to determine paternity/maternity (and other familial relationships). The best-known application of DNA fingerprinting is in forensic science. DNA fingerprinting techniques are utilized to interpret blood, tissue, or fluid evidence collected at accidents and crime scenes. After DNA is extracted from these samples, forensic scientists can develop a DNA fingerprint. The DNA fingerprint from a crime scene can then be compared to the DNA fingerprints of different suspects. A match provides strong evidence that the suspect was present at the crime scene.

Experiment Overview



Gel pattern will vary depending upon the experiment.

Agarose Gel Electrophoresis



IMPORTANT:

If you are unfamiliar with agarose gel prep and electrophoresis, detailed instructions and helpful resources are available at **www.edvotek.com**

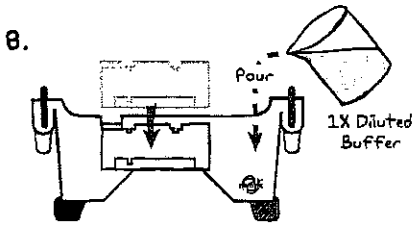
Wear gloves and safety goggles

1. **DILUTE** concentrated (50X) buffer with distilled water to create 1X buffer (see Table A).
2. **MIX** agarose powder with 1X buffer in a 250 ml flask (see Table A).
3. **DISSOLVE** agarose powder by boiling the solution. **MICROWAVE** the solution on high for 1 minute. Carefully **REMOVE** the flask from the microwave and **MIX** by swirling the flask. Continue to **HEAT** the solution in 15-second bursts until the agarose is completely dissolved (the solution should be clear like water).
4. **COOL** agarose to 60° C with careful swirling to promote even dissipation of heat.
5. While agarose is cooling, **SEAL** the ends of the gel-casting tray with the rubber end caps. **PLACE** the well template (comb) in the appropriate notch.
6. **POUR** the cooled agarose solution into the prepared gel-casting tray. The gel should thoroughly solidify within 20 minutes. The gel will stiffen and become less transparent as it solidifies.
7. **REMOVE** end caps and comb. Take particular care when removing the comb to prevent damage to the wells.

Table A — Individual 0.8% UltraSpec-Agarose™ Gel

Size of Gel Casting tray	Concentrated Buffer (50x)	+ Distilled Water	+ Amt of Agarose	= TOTAL Volume
7 x 7 cm	0.6 ml	29.4 ml	0.23 g	30 ml
7 x 10 cm	1.0 ml	49.0 ml	0.39 g	50 ml
7 x 14 cm	1.2 ml	58.8 ml	0.46 g	60 ml

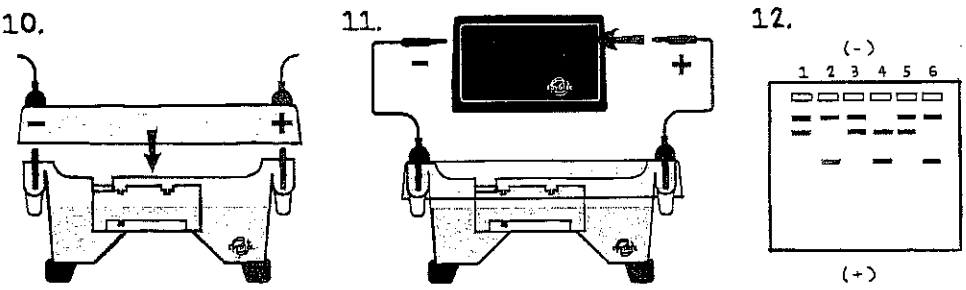
Agarose Gel Electrophoresis



Reminders:

If unfamiliar with gel loading, consider performing the optional activity in Appendix C, Practice Gel Loading, prior to performing the experiment.

Before loading the samples, make sure the gel is properly oriented in the apparatus chamber.



8. **PLACE** gel (on the tray) into electrophoresis chamber. **COVER** the gel with 1X electrophoresis buffer (See Table B for recommended volumes). The gel should be completely submerged.
9. **PUNCTURE** the foil overlay of the QuickStrip™ with a pipet tip. **LOAD** the entire sample (35-38 µL) into the well in consecutive order. The identity of each sample is provided in Table 1.
10. **PLACE** safety cover. **CHECK** that the gel is properly oriented. Remember, the DNA samples will migrate toward the positive (red) electrode.
11. **CONNECT** leads to the power source and **PERFORM** electrophoresis (See Table C for time and voltage guidelines).
12. After electrophoresis is complete, **REMOVE** the gel and casting tray from the electrophoresis chamber and **VISUALIZE** the results. No staining is necessary.

Table 1: Gel Loading

Lane		
1	Tube A	Standard Dye Marker
2	Tube B	Dye Sample B
3	Tube C	Dye Sample C
4	Tube D	Dye Sample D
5	Tube E	Dye Sample E
6	Tube F	Dye Sample F

Table B — 1x Electrophoresis Buffer (Chamber Buffer)

EDVOTEK Model #	Total Volume Required	Dilution: 50x Conc. Buffer	+ Distilled Water
M6+ & M12 (new)	300 ml	6 ml	294 ml
M12 (classic)	400 ml	8 ml	392 ml
M36	1000 ml	20 ml	980 ml

Table C — Time and Voltage Guidelines (0.8% Agarose Gel)

	Electrophoresis Model: M6+	M12 (new)	M12 (classic) & M36
Volts	Min. / Max.	Min. / Max.	Min. / Max.
150	15/20 min.	20/30 min.	25 / 35 min.
125	20/30 min.	30/35 min.	35 / 45 min.
75	35 / 45 min.	55/70 min.	60 / 90 min.