

TEACHER'S GUIDE – PART 2

DNA FINGERPRINTING SIMULATION

Gel Electrophoresis

The term *electrophoresis* literally means, "to carry with electricity." It is a technique for separating and analyzing mixtures of charged molecules. Because of its sugar-phosphate backbones, DNA is a negatively-charged molecule. When DNA is placed in an electric field, it will migrate toward the positive electrode (anode). The speed of migration of DNA in an agarose gel depends on the size of the piece. Small pieces experience less resistance when moving through the mesh created by the agarose gel and move faster than the larger pieces. Thus, small pieces of DNA migrate farther in the gel than large pieces and are found closer to the bottom of the gel.

Equipment and Supplies used in today' Lab

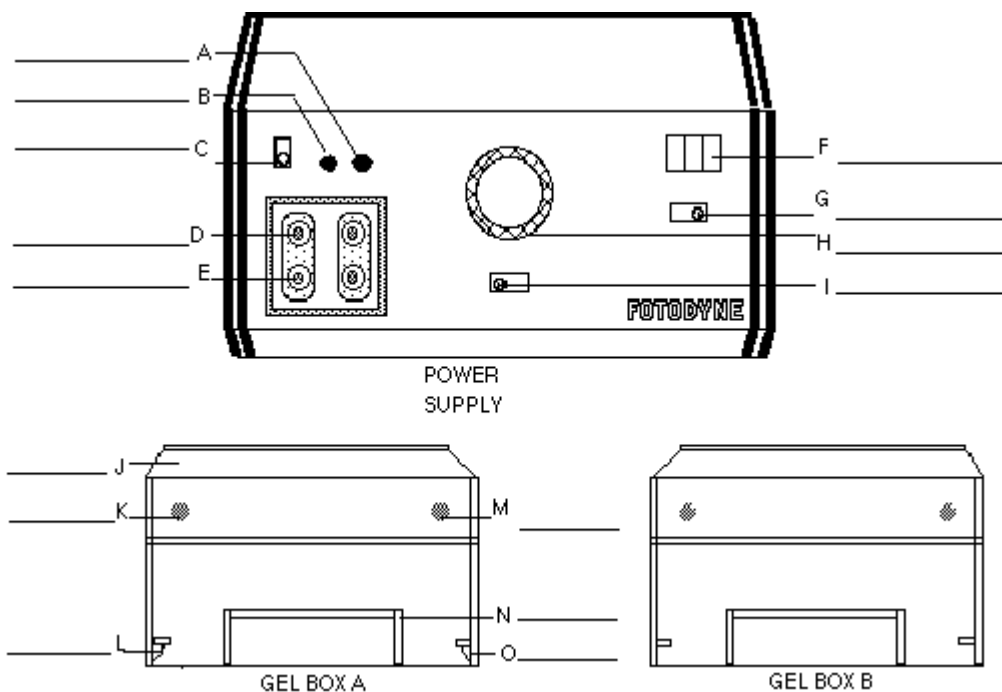
For each group

- Gel electrophoresis box
- Power supply
- Casting tray and comb
- 1-20 μ l Micropipettor
- pipette tips for Micropipettor
- racks for 1.5 ml microcentrifuge tubes
- 1.5 ml microcentrifuge tubes
- plastic tray for hold gels when moving them
- beakers for 1X TAE buffer
- marking pen
- plastic wrap
- ice buckets
- container for waste

For the class

- microcentrifuge

Gel Electrophoresis Apparatus Diagram



Labels for the Gel Electrophoresis Apparatus

On power supply

digital display
on/off switch
voltage select dial
switch between Volts and milliAmps
(-) and (+) channels for one of the gel boxes
lo/hi range switch (on Lo 20-160 V; on Hi, 165-300 V)
platform for casting tray
light indicating "POWER SUPPLY IS ON"
light indicating "CHECK FUSE"

and

red & black wires connecting power supply with each gel box

On each gel box

anode
cathode
(+) lead
(-) lead
lid

Reagents used in the Lab

1. **TAE electrophoresis buffer** – buffer for the gel electrophoresis boxes. You can reuse it. You can prepare a 50X stock buffer that can then be diluted to 1X to use in the gel electrophoresis box. Dilute with distilled water. You'll need 5-10 liters of 1X TAE.

50X TAE Stock Solution, pH ~ 8.5
242 gm Tris base
57.1 mL glacial acetic acid
37.2 gm Na₂EDTA x 2H₂O
Add H₂O to one liter

At 1X this is 40 mM Tris acetate, 2 mM EDTA
1X TBE buffer can be used in place of TAE buffer.

1 X TBE
100 mM Tris
100 mM H₃BO₃
1 mM EDTA, pH 8.3

2. **10x Agarose Gel Loading Dye** – the dye is added to the DNA samples before loading the gel to make them dense and sink to the bottom of the well.

15% Ficoll, 0.2% bromophenol blue, 0.2% xylene cyanol FF in distilled water.

1.5 g Ficoll (Sigma F-2637)
0.02 g Bromophenol blue (Sigma B-0126)
0.02 g xylene cyanole FF (Kodak T-1579)
add distilled H₂O to 10 ml (store at -20°C).

Technical Skills to Teach the Students

This lab requires that the students learn how to use a micropipettor, sterile technique and balance the tubes in the rotor when using a microcentrifuge. Ask if they know what sterile technique is. Listen to their answers, and then explain that sterile technique is method of working where the environment is essentially bacteria-free so that there is no contamination. This technique is required in tomorrow's experiment because the restriction enzymes and DNA they will be using are susceptible to degradation by enzymes in the environment. Thus, the microcentrifuge tubes and tips (for the pipettor) will be sterilized. Their job is to open the container that contains the tips and remove them with the pipettor and use them without touching any surfaces such as the lab bench, their hands or the tip box. Pipetting properly is essential as well. If they do not use the pipettor properly, the solution will enter the body of the pipettor and be lost. In addition, they will not have enough of the reagents they will be using for the experiment. Also accuracy is important when trying to get good scientific results, so pipetting carefully is a necessity. Bring



sterile microcentrifuge tubes and tips to the classroom to show them. Also bring a 1-20 μ l pipettor and demonstrate how they are to use it. If time allows, let them practice by pipetting water from a beaker into another beaker. In addition, balancing the rotor of a centrifuge is essential or it not rotate correctly. Explain that they must put their samples in the rotor as if they were a pair. Each pair must contain the same amount of solution and be placed across from each other in the rotor. They will also run an electrophoresis gel, then stain and photograph it. Be prepared to demonstrate all of these skills and techniques.

