

SEPARATION OF DNA USING AGAROSE GEL ELECTROPHORESIS

MATERIALS:

Electrophoresis apparatus and power supply
Agarose (0.2 gram in 2 ml Eppendorf tube – pre-measured)
125 ml Erlenmeyer flask
50 cc centrifuge tube for measuring the TAE buffer
1X TAE buffer
micropipette (0-20 ul)
micropipette tips (yellow)
paper towels

METHOD:

1. To make a 0.8% agarose gel:

- Transfer the 0.2 grams of agarose into your 125ml Erlenmeyer flask.
- Measure **25 ml** 1X TAE buffer (bottle with green tape) using the 50 cc centrifuge tube and add to the agarose in the Erlenmeyer flask. Swirl gently to mix.
- Microwave for 20 seconds. Check to see if all the particles are dissolved and the solution is very hot. If not, heat again for no more than ten seconds.
- Bring to your station and let cool until it is lukewarm to the touch. To help the agarose cool, swirl it gently in the flask. If the agarose is too hot, it will leak out the ends of the red striped gel tray.
- **IMPORTANT!!!** When the agarose has cooled to lukewarm, add **two drops** of the dye in the small white bottles. This is Carolina Blue Dye. If you do not add this dye to the gel before you pour it into the red striped tray, you will NOT be able to see the DNA.
- While the gel is cooling, place the black dams in the gel box on both ends of the gel tray insert. Be sure the flat side of the dams are against the gel tray insert. The tops of the dams should be perfectly flat if they are put in correctly.
- Insert the **8-well comb** in the red striped tray in the slot nearest the cathode.
- When the agarose has cooled to the point that you can hold the bottom of the Erlenmeyer flask comfortably, *and after you have added the FastBlast Dye*, pour the 25 ml of agarose into the gel tray.
- Let the gel harden for at least 10 minutes. Do not move the tray while setting.
- Once the agarose is hardened, remove the two black dams.
- **Add 125 ml of 1X TAE Buffer (bottle with green tape)** – an amount sufficient to just cover the gel.
- Carefully “wiggle” the comb back and forth to separate the comb from the gel and then lift *slowly and gently* out of the gel.

Gel Electrophoresis Analysis of DNA Samples

You have been provided with **prealiquoted** DNA in three marked eppendorf tubes. Two more solutions must be added to these DNA samples to prepare them for electrophoresis. First, a **Restriction Enzyme (RE)** must be added to cut the DNA molecule at very specific sites into different length pieces. Then you will need to add **Loading Dye (LD)** to give color to the clear DNA. This dye will move ahead of the DNA so you can determine when the electrophoresis is finished.

- Pipette the required amount of Restriction Enzyme into each tube. If you touch the DNA sample with the RE pipette tip, be sure and change tips so you don't contaminate any other sample.
- Pipette the required amount of Loading Dye into each of the three test tubes. **BE SURE AND CHANGE YOUR MICROPIPETTE TO 2ul** before adding the loading dye.

Prealiquoted DNA Sample	Restriction Enzyme	Loading Dye
#1 (6 μ l)	5 μ l	2 ul
#2 (6 μ l)	5 μ l	2 ul
#3 (6 μ l)	5 μ l	2 ul

- Mix the contents of the tubes by centrifugation using a microcentrifuge. Be sure your tubes are balanced! Close the lid and depress the start button on the back of the unit. Centrifuge for three seconds.

Loading the Samples

Use the micropipettes as demonstrated previously by the instructor. Set to 13 μ l. Each well has a bottom made of a very thin layer of gel. It is very easy to break through and ruin your gel. Be sure and brace your arms and hands while pipetting! The Loading Dye contains glycerol, a heavy material which helps to carry the DNA sample to the bottom of the well. However, sometimes very small amounts of sample will "float" out of the wells. These small amounts of sample will be diluted in the buffer and will not affect the experiment.

- Carefully pipette 13 μ l of Sample #1 into a well.
- Skip a well.
- Carefully pipette 13 μ l of Sample #2 into a well.
- Skip a well.
- Carefully pipette 13 μ l of Sample #3 into a well.

Running the Samples

- Set the range switch on the power supply to LOW and the voltage between 110-120V.
- You will be able to see tiny blue DNA bands forming behind the loading dye as it moves through the gel. Run your samples until you feel you can determine a clear difference between the samples.

Removing Gel from the Electrophoresis Box

- Turn the power supply off and disconnect the cables.
- Gently lift the red stripe casting tray with the gel out of the box. Do NOT put your gel onto a paper towel!
- Slide the gel onto the light box for observation of banding patterns.

NAME _____ PARTNER _____

Data/Observation Sheet

Agarose Gel Preparation:

1. What is the purpose of the agarose gel?

What is the purpose of the blue dye that was added to the agarose gel?

DNA Preparation:

1. What is the function of the Restriction Enzyme?

2. What purpose does the Loading Dye serve?

Gel Analysis:

1. Which DNA sample matches the DNA taken from the scene of the crime?

2. Explain why there are differences and similarities in the DNA banding patterns.