

# SEPARATION OF MOLECULES USING AGAROSE GEL ELECTROPHORESIS

## Biotechnology Workshop

### Materials

Electrophoresis apparatus and power supply  
Agarose (0.25 grams in 2 ml eppendorf tube--prealiquoted)  
125 ml Erlenmeyer flask  
Set of dyes (in marked 0.5 ml eppendorf tubes)  
150 ml 1X TAE buffer  
micropipette (0-20  $\mu$ l)  
micropipette tips (yellow)

### Methods

#### 1. To make a 1.0% agarose gel:

- Transfer the 0.25 grams of agarose into a clean 125 ml Erlenmeyer flask.
- Add 25 ml 1X TAE buffer.
- Microwave on HIGH for 20 seconds. Check to see if all the particles are dissolved and solution is starting to boil. If not, heat again for no more than 10 seconds.
- Make a "paper towel handle" to remove the flask from the microwave. BE CAREFUL. It will be extremely hot!!
- Place dams in the gel box on both ends of the gel tray insert. Be sure that the flat side of the dams are against the gel tray insert.
- Pour the 25 ml of agarose into the gel tray. Insert the 8-well comb in the center of the gel tray.
- Let the gel harden for at least 10 minutes. Do not move while setting.
- After the gel has set, add a small amount of 1X TAE to the top of the gel. This will facilitate removal of the comb. Removing the comb is a critical step and must be done carefully to prevent tearing of the wells. Rub a finger gently along the edge of the comb on both sides to loosen it. Then CAREFULLY pull the comb straight up to prevent tearing of the wells.
- Remove the dams.
- Add 125 ml of 1X TAE--an amount sufficient to just cover the gel.

## 2. Adding the dye samples

You have been given 7 dyes and one unknown sample.

- Carefully pipette **10  $\mu$ l** of each of the dyes into the wells starting with the first lane. Change your pipette tip with each dye.
  - \* One of the most common mistakes is not bracing arms or hands while pipetting. This often results in pushing the pipette through the bottom of the well, resulting in dispersion of the dye under the gel.
  - \* Very small amounts of the dyes will inevitably 'float' out of the wells. These small amounts of dye will be diluted in the buffer and will not affect the running of the dyes that remain in the wells.
  - \* Remember to write down the order in which you pipette the dyes.

## 3. Running the dye samples

- After all the dyes have been pipetted into the gel wells, close the lid, attach the power cords, set the range switch to LOW and the voltage to 100V.
- Run the gel for approximately 10 minutes or until you get good separation of all of the dyes--especially the unknown which contains several dyes.

## 4. Recording of results

- Turn the electrophoresis unit off and carefully remove the gel tray.
- Record the direction of travel (to cathode or anode) and distance that each of the dyes has traveled. Compare the distance and color match of each of the known dyes to the unknown (E2) to determine what dyes are contained within the unknown.
- You can also have a permanent record of your results by doing a "blot" on a paper towel!