

## **Agarose Gel Electrophoresis Protocol**

### **Preparing the agarose gel:**

- For a small gel, measure 40 mL of TBE or TAE buffer in a graduated cylinder and pour it into a 250 mL flask.
- Weigh 0.4 grams of electrophoresis grade agarose and add it to the buffer solution in the flask and swirl it gently.
- Melt the agarose by heating it in a microwave or hot water bath.
- Cool the agarose to about 50-55°C in a water bath for about 5-10 minutes (until it doesn't burn the surface of your inner forearm). Swirl the agarose occasionally while it cools.
- Place the gel tray into the gel box so that the ends of the tray are blocked by the sides of the box (perpendicular to the ends of the box).
- Place the comb in the slots on one end of the tray.
- Pour the cooled agarose into the tray and let it cool until it is solid (it should appear milky white).
- Carefully pull upward on the comb to remove it once the agarose has solidified.
- Remove the tray from the box and replace it so that the ends of the gel are open to the chambers on either side of the gel box.
- Pour buffer solution into the gel box so that there is an equal amount of buffer solution in either chamber and so there is about 2-3 mm of solution above the gel.

### **Loading the gel:**

- Place 2 µl of loading dye onto a square of Parafilm.
- Pipette 8 µl of your PCR reaction onto this 2 µl spot of loading dye and slowly pipette the mixture up and down to mix it.
- Reset your micropipette to 10 µl and carefully pipette the PCR/loading dye mixture into a well of your gel.
- Pipette 10 µl of DNA ladder into another well of the gel.
- Draw a map in your laboratory notebook with labels to indicate which well contains your PCR products as well as the DNA ladder.

### **Running the gel:**

- Connect the lid to the gel box (make sure that the negative pole is on the same side as the wells of your gel!) and then connect the positive and negative leads into the appropriate connection on the power supply.
- Turn the power supply on and adjust the voltage to about 120 volts.
- Check that the loading dye is running toward the correct end of your gel after a few minutes.
- Run the gel until the first dye is near the end of your gel.
- Switch the power supply off, remove the lid, and carefully lift your gel tray out.

### **Staining and photographing the gel:**

- Carefully place your gel into the staining tray with the gel stain (ethidium bromide).
- Let the gels stain for 10-20 minutes.
- Carefully remove the gel. place it in a glass dish and rinse it 2-3 times with distilled water.
- Use a spatula to place the gel on the UV light source and photograph it.
- Dispose of the gel in the biohazard waste container when finished.