Exp.20 Agarose Gel Electrophoresis

Introduction:

Agarose gel electrophoresis is used to separate DNA or RNA based on charge, size and shape by moving negatively charged nucleic acid molecules through agarose matrix toward positive pole under electric field.

Factors affecting the migration rate: the percent agarose in the gel, the length and conformation of the molecule, buffer, applied voltage.

After the electrophoresis is complete, the gel could be illuminated under UV light to make DNA or RNA bands visible, using ethidium bromide (abbreviated as EtBr). It fluoresces reddish-orange when it intercalated into the major groove of DNA or RNA. EtBr is a mutagen. Finally, the gel is photographed with a digital or polaroid camera. The DNA or RNA band can also be cut out of the gel, and then be dissolved to retrieve the purified molecules. Three common EtBr staining include:

(1) Adding 0.5 μg/ml directly to Agarose gel

(2) Adding 0.4 μg/ml directly into the sample and loading on EtBr-free gel

(3) Staining the gel after completion of electrophoresis

The molecular size is determined by relating to the linear relationship of log (MW) v.s. Rf of DNA/RNA marker.

Reagents:

1. Agarose
2. Glycerol (to make samples dense)

3. TBE Buffer
4. EtBr (5 mg/mL)

Procedure:

1. Preparation 0.7% of Agarose Gel

Add 0.14 g of Agarose to 125 ml Erlenmeyer flask. Add 20 ml of the 0.5x TBE buffer and swirl to suspend the Agarose powder in the buffer.

(Note: A mark can be put on the flask at the same level as the liquid. If evaporation occurs, water can be added to the original starting level.)

Place the flask into the microwave oven until all the small Agarose particles are dissolved. Cool the molten Agarose to 60 °C, and add 2µl of EtBr. (Caution: Always wear protective gloves, goggles, and a lab coat.)

2. Casting Agarose Gel

Level the Mini-Gel Caster. Place the open edge of the tray against the fixed wall of the Caster. Place the comb into the appropriate slot of the tray. Pour the 50-60 °C Agarose on the tray immediately. Avoid bubbles. After ~20min, the gel has solidified, remove the comb carefully.

3. Setup and Run Electrophoresis

Place the tray on the platform of gel electrophoresis apparatus, and the wells are at the negative end. Add buffer to cover the gel

over 2-3 mm. Use pipets to load the samples containing loading dye for underlaying into sample wells.

After the samples are loaded, carefully snap the cover down onto the electrode terminals. Insert the plug into the power source. Set the power source at 100 volts and run the electrophoresis for 30~50 minutes.

Results：

1. Record your observations.
2. Label the molecular weight of each band.

Discussions：