

## BT 510 Analytical Biotechnology Lab

### **ANALYSIS OF DNA BY GEL ELECTROPHORESIS**

#### **MATERIALS:**

- a. Agarose
- b. TAE buffer (50 X)
- c. Loading dye (6 X)
- d. Gel running boat
- e. Minigel apparatus and power supply
- f. Ethidium bromide stock solution ( $10 \text{ mg ml}^{-1}$ )
- g. Sterile microcentrifuge tube, 1.5 ml
- h. Sterile microtips

#### **WORKING PROTOCOL:**

##### **I. AGAROSE GEL ELECTROPHORESIS**

1. Seal the gel running boat and place the comb for the wells.
2. Add 0.8 g agarose to 100 ml 1 x TAE buffer. Heat the mixture in a microwave oven to dissolve agarose.
3. Allow the solution to cool to around  $45^{\circ}\text{C}$  and pour into the sealed boat.
4. Allow the gel to polymerize. Remove the comb after the gel polymerizes and place the gel along with the boat in the electrophoresis tank.
5. Add sufficient volume of 1 x TAE buffer in the tank to cover the surface of the gel.
6. Mix  $4 \mu\text{l}$  of each sample with  $2 \mu\text{l}$  of loading dye and  $6 \mu\text{l}$  of sterile water in a thin strip of parafilm on the working bench. Load the samples on gel.
7. Set the voltage to 50-60 volts and perform electrophoresis of the samples till the dye migrates  $3/4$ th of the gel.
8. Remove the gel from the tank and stain by soaking in Ethidium bromide solution ( $0.5 \mu\text{g ml}^{-1}$ ) for 10 min at room temperature.
9. Observe in a CCD-based gel documentation system.

## **BUFFERS AND REAGENTS:**

### **A. 70% ALCOHOL**

### **B. TAE BUFFER (50 X):**

24.2 g Tris base, 5.71 ml of glacial acetic acid and 10 ml of 0.5 M EDTA (pH 8.0) was added to 50 ml of ultrafiltered water. The pH of the buffer was adjusted to 7.2 and the final volume to 100 ml with ultrafiltered water. The buffer was sterilized by autoclaving and stored at room temperature.

### **C. LOADING DYE (6 X):**

#### *Ingredients Quantity*

Bromophenol blue 0.25% (w/v)

Xylene cyanol 0.25% (w/v)

Glycerol 30% (v/v)

The above components were dissolved in ultrafiltered water, dispensed in aliquots and stored at 4°C.

### **D. ETHIDIUM BROMIDE STOCK SOLUTION (10 mg ml<sup>-1</sup>):**

10 mg of Ethidium bromide was dissolved in 1 ml of ultrafiltered water. The solution was stored in a microcentrifuge tube, wrapped in aluminum foil and stored at 4°C.

**Caution: Ethidium bromide is a carcinogen and should always be handled wearing gloves.**