OBJECTIVE: Agarose gel electrophoresis

Chemicals required: LE Agarose (EEO: 0.09-0.13), ethidium bromide, tris base, EDTA, glacial acetic acid, bromophenol blue, sucrose, ddH_2O/ Milli Q, NaOH, HCl.

Plastic ware/glassware: measuring cylinder, autoclaved micro centrifuge tubes, 1.0 ml tips, 0.02ml-0.2 ml tips, enzyme tips, 100ml/250 ml flasks, reagent bottle.

Equipments required: Horizontal electrophoresis unit, power supply, casting tray, combs, pH meter.

Recipe for buffer

1. **50X TAE (200 ml):** 48.4 g of Tris base, 7.44 g of EDTA ,11.42 ml of glacial acetic acid
2. Volume make up to 200 ml by Milli Q. pH adjusted to 8.0.
3. **6X Loading dye**: glycerol 60%, Tris-HCl (pH 7.6) 10 mM, EDTA 60 mM, bromophenol blue 0.03%, xylene cyanol 0.03%.
4. **Ethidium bromide(10mg/ml):** weigh 10 mg ethidium bromide and add 1ml ddH2O/milliQ. Allow it to mix properly (use orbital shaker).

PROTOCOL

1. Prepare 1% agarose gel (0.5g in 50 ml 1X TAE), boil it until it dissolves. Cool to RT and add ethidium bromide to final concentration of 1 µg/ml.
2. Cast gel by pouring it into a casting tray with a comb placed in it.
3. Let the gel solidify, place the tray in buffer tank and remove comb.
4. Load plasmid DNA (2µl plasmid DNA+1µl 6X loading dye+ 3 µl MilliQ) adjacent to the ladder.
5. Apply voltage of 5-8 V/cm. Visualize the DNA bands under UV light.