**Methods of Agarose Gel Electrophoresis**

* **Prepare a 50x stock solution of TAE buffer in 1000m of distilled H2O:**
* For this weigh 242 g of Tris base in a chemical balance. Transfer this to a 1000ml beaker.
* Prepare EDTA solution (pH 8.0, 0.5M) by weighing 9.31g of EDTA and dissolve it in 40ml distilled water. EDTA is insoluble and it can be made soluble by adding sodium hydroxide pellets. Check the pH using pH meter. Make the solution 100ml by adding distilled water.
* Pipette out 57.1 ml of glacial acetic acid.
* Mix the Tris base, EDTA solution and glacial acetic acid and add distilled water to make the volume to 1000ml
* **Prepare sufficient electrophoresis buffer (usually 1x TAE ) to fill the electrophoresis tank and to cast the gel:**
* **Prepare a solution of agarose in electrophoresis buffer at an appropriate concentration:**
* Heat the slurry in a boiling-water bath or a microwave oven until the agarose dissolves. The agarose solution can boil over very easily so keep checking it. It is good to stop it after 45 seconds and give it a swirl. It can become superheated and NOT boil until you take it out whereupon it boils out all over you hands. So wear gloves and hold it at arm's length. You can use a Bunsen burner instead of a microwave - just remember to keep watching it.
* Use insulated gloves or tongs to transfer the flask/bottle into a water bath at 55°C. When the molten gel has cooled, add 0.5µg/ml of ethidium bromide. Mix the gel solution thoroughly by gentle swirling.
* **While the agarose solution is cooling, choose an appropriate comb for forming the sample slots in the gel.**
* Pour the warm agarose solution into the mold.
* Allow the gel to set completely (30-45 minutes at room temperature), then pour a small amount of electrophoresis buffer on the top of the gel, and carefully remove the comb. Pour off the electrophoresis buffer. Mount the gel in the electrophoresis tank.
* Add just enough electrophoresis buffers to cover the gel to a depth of approx. 1mm.
* Mix the samples of DNA with 0.20 volumes of the desired 6x gel-loading buffer.
* Slowly load the sample mixture into the slots of the submerged gel using a disposable micropipette or an automatic micropipettor or a drawn-out Pasteur pipette or a glass capillary tube. Load size standards into slots on both the right and left sides of the gel.
* Close the lid of the gel tank and attach the electrical leads so that the DNA will migrate toward the positive anode (red lead). Apply a voltage of 1-5 V/cm (measured as the distance between the positive and negative electrodes). If the electrodes are 10cm apart then run the gel at 50V. It is fine to run the gel slower than this but do not run it any faster. Above 5V/cm the agarose may heat up and begin to melt with disastrous effects on your gel's resolution. If the leads have been attached correctly, bubbles should be generated at the anode and cathode.
* Run the gel until the bromophenol blue and xylenecyanol FF have migrated an appropriate distance through the gel.
* The gel tray may be removed and placed directly on a transilluminator. When the UV is switched on we can see orange bands of DNA.