### 3.1.1.8. Agarose gel electrophoresis of DNA

Nucleic acid can be separated electrophoretically according to their size. This separation is done on agarose or polyacrylamide gels. Movement of charged particles in an electric field is called electrophoresis. Thus electrophoresis is migration of a charged molecule in an electric field in the direction of an electrode so that cations (positively charged molecules) move in the direction of cathode (negative electrode) and anions (negatively charged molecules) move in the direction of anode (positive electrode). When an electric field is applied to an agarose gel in the presence of a buffer solution which will conduct electricity, DNA which is highly negatively charged at neutral pH, will migrate towards the positive electrode at rates determined by their molecular size and conformation. Electrophoresis with agarose as the support medium is the standard method for the characterization of RNA and DNA of 200-50, 000 bp. Agarose is extracted from seaweed and is a linear polymer of D-galactose and 3,6-anhydro-L-galactose. Porosity of the agarose gels is determined by the concentration of the agarose. Higher the agarose concentration, smaller is the pore size and lesser the agarose concent-

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ration, larger the pore size. Nucleic acids can be visualized on agarose gels after separation by soaking in a solution of ethidium bromide, a dye that fluoresces under UV light and intercalates between stacked nucleic acids. Most commonly agarose concentrations of 0.3-2% are used for nucleic acid separation. The movement of a charged molecule subjected to an electric field is given by,

$$v = \frac{Eq}{f}$$

where,

E= the electric field in volts/cm

q= the net charge on the molecule

f= frictional coefficient, depending on the mass and charge of the molecule

v= the velocity of the molecule.

The movement of charged molecules in an electric field is often expressed in terms of electrophoretic mobility (M) which is defined as velocity per min. of electric field.

$$\mu = v/E = E.q/\ f.E \text{ (since } = E.q \backslash f) = q/f$$

Thus, the electrophoretic mobility ($\mu$) of a molecule is directly propotional to the charge density (charge\mass ratio). Therefore, molecules with different charge\mass ratio migrate at different rates under an electric field and get separated.

The charge\mass ratio of nucleic acids in one, therefore, rate of migration of DNA is inversely proportional to $\log_{10}$ of their molecular weights. Thus smaller DNA molecules will migrate at faster rates than larger ones.

**Factors affecting the rate of migration in agarose gel:**

1. Molecular size of DNA: Migration rate of linear ds DNA is inversely proportional to $\log_{10}$ of the number of base pairs.
2. Agarose concentration: There is a linear relationship between the logarithm of the electrophoretic mobility of DNA ($\mu$) and the gel concentration (x)

$$\text{Log } \mu = \log \mu_0 - K_r x$$

3. Conformation of DNA: Rate of movement is based on the conformation of DNA as follows,

Superhelical form > nicked circular form > linear DNA

4. Applied Voltage: At low voltage rate of migration of linear DNA fragment is proportional to applied voltage.

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5. Presence of intercalating dye: EtBr reduces the electrophoretic mobility of linear DNA by about 15%.
6. Composition of electrophoretic buffer: DNA migrates very slowly in presence of low ionic strength buffer while in high ionic strength buffer heat is generated which may melt the gel.

Procedure

1. Prepare sufficient amount of electrophoresis buffer to fill the tank (600 ml).
2. Weigh 1 gm agarose and dissolve in 100 ml 1X TAE buffer (1% agarose solution).
3. Heat the contents in boiling water bath till agarose dissolves.
4. Allow the contents to cool to 60°C.
5. Add 3-5 μl of EtBr to the gel.
6. Pour the agarose solutions into the mould.
7. Gel should be 3-5 mm thick, allow the gel to set.
8. Remove the comb from the gel.
9. Pour the buffer into the electrophoresis tank. The level of buffer should be 1 ml above the gel.

Loading of samples

1. Add 6 μl of 6X Sample Loading Buffer to each sample (2:4).
2. Loading buffers are added with the DNA in order to visualize it and sediment it in the gel well. Negatively charged indicators keep track of the position of the DNA. Xylene cyanol and Bromophenol blue are typically used. They run at about 500bp and 300bp respectively, but the precise position varies with percentage of the gel. Other less frequently used progress markers are Cresol Red and Orange G which run at about 125bp and 50bp.
3. Record the order of each sample that will be loaded on the gel, the DNA template - what organism the DNA came from, controls and ladder.
4. Carefully pipette 5-15 μl of each sample/sample loading buffer mixture into separate wells in the gel.
5. Pipette 1-2 μl of the DNA ladder standard into at least one well of each row on the gel.

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## Running of the gel

1. Place the lid on electrophoresis chamber, connecting the electrodes.
2. Connect the electrode wires to the power supply, making sure the positive (red) and negative (black) are correctly connected.
3. Turn on the power supply to about 100 volts. Maximum allowed voltage will vary depending on the size of the electrophoresis chamber – it should not exceed 5 volts/cm between electrodes.
4. Check to make sure the current is running through the buffer by looking for bubbles forming on each electrode.
5. Check to make sure that the current is running in the correct direction by observing the movement of the blue loading dye. This will take a couple of minutes (it will run in the same direction as the DNA).
6. Let the power run until the blue dye approaches the either end of the gel.
7. Turn off the power.
8. Disconnect the wires from the power supply.
9. Remove the lid of the electrophoresis chamber.
10. Using gloves, carefully remove the tray and gel.

## Visualizing the gel

Using gloves, remove the gel from the casting tray. As EtBr was added to the gel that will intercalate with the DNA bands and make them fluoresce, DNA bands can be visualized using UV illuminator. Care must be taken to avoid UV exposure to the body. A Gel Documentation system can also be used to visualise the DNA bands and a monitor may be connected to the system that will make easy data recording and storage.

## Precautions

1. Gel should be completely immersed in TAE buffer.
2. Proper loading of samples should be done and the wells should not over flow.
3. Gel should be run at proper voltage and current.
4. Gloves should be worn when handling ethidium bromide.
5. Proper protection of eyes should be taken when visualizing the DNA bands under UV light.

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