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ELECTROPHORESIS OF AGAROSE GEL ASSISTING SEPARATION OF DNA FRAGMENTS

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ABSTRACT

This review describes the electrophoresis of curved and normal DNA molecules in agarose gels, polyacrylamide gels and in free solution. These studies were undertaken to clarify why curved DNA molecules migrate anomalously slowly in polyacrylamide gels but not in agarose gels. Two milestone papers are cited, in which Ferguson plots were used to estimate the effective pore size of agarose and polyacrylamide gels. Subsequent studies on the effect of the electric field on agarose and polyacrylamide gel matrices, DNA interactions with the two gel matrices, and the effect of curvature on the free solution mobility of DNA are also described. The combined results suggest that the anomalously slow mobilities observed for curved DNA molecules in polyacrylamide gels are due primarily to preferential interactions of curved DNAs with the polyacrylamide gel matrix.Curved DNA molecules migrate anomalously slowly in free solution as well as in polyacrylamide gels, explaining why the Ferguson plots of curved and normal DNAs containing the same number of base pairs extrapolate to different mobilities at zero gel concentration.

Introduction :-

Agarose gel electrophoresis is the most effective way of separating DNA fragments of varying sizes ranging from 100 bp to 25 kb.Agarose polymers associate non-covalently and form a network of bundles whose pore sizes determine a gel's molecular sieving properties. The use of agarose gel electrophoresis revolutionized the separation of DNA. Gal electrophoresis method was developed in the 1960s and 1970s. Starch, agar, or polyacrylamide were originally used as the gel matrix. In 1973, Joseph Sambrook and colleagues at the Cold Spring Harbor Laboratory pioneered the use of agarose, a highly purified polysaccharide extracted from seaweed. DNA gel electrophoresis requires the use of specialized apparatus, toxic reagents, expensive agarose gel, and DNA samples, as well as a considerable amount of valuable classroom time to complete.

Argarose gel electrophoresis may be employed effectively for the detection and preliminary characterization of plasmid deoxyribonucleic acid (DNA) present in clinical isolates and laboratory strains of gram-negative microorganisms. Agarose (containing vicinal hydroxyl) group interacting with borates. agarose is reported to compete for binding, rendering the agarose interaction dominant over the DNA interaction.

Electrophoresis is the migration of charged particles or molecules in an electric field. This occurs when the substances are in aqueous solution. The speed of migration is dependent on the applied electric field strength and the charges of the molecules. Thus, differently charged molecules will form individual zones while they migrate.

Method Of Prepare Agarose Gel Electrophoresis

1) Preparation of Gel:-

1. Weigh out the appropriate mass of agarose into an Erlenmeyer flask. Agarose gels are prepared using a w/v percentage solution. The concentration of agarose in a gel will depend on the sizes of the DNA fragments to be separated, with most gels ranging between 0.5%-2%. The volume of the buffer should not be greater than 1/3 of the capacity of the flask.

2. Add running buffer to the agarose-containing flask. Swirl to mix. The most common gel running buffers are TAE (40 mM Tris-acetate, 1 mM EDTA) and TBE (45 mM Tris-borate, 1 mM EDTA).

3. Melt the agarose/buffer mixture. This is most commonly done by heating in a microwave, but can also be done over a Bunsen flame. At 30 s intervals, remove the flask and swirl the contents to mix well. Repeat until the agarose has completely dissolved.

4. Add ethidium bromide (EtBr) to a concentration of 0.5 µg/ml. Alternatively, the gel may also be stained after electrophoresis in running buffer containing 0.5 µg/ml EtBr for 15-30 min,

followed by destaining in running buffer for an equal length of time.

5. Allow the agarose to cool either on the benchtop or by incubation in a 65 °C water bath. Failure to do so will warp the gel tray6. Place the gel tray into the casting apparatus. Alternatively, one may also tape the open edges of a gel tray to create a mold. Place an appropriate comb into the gel mold to create the wells.

7. Pour the molten agarose into the gel mold. Allow the agarose to set at room temperature. Remove the comb and place the gel in the gel box. Alternatively, the gel can also be wrapped in plastic wrap and stored at 4 °C until use (Fig. 1).

2) Setting up of Gel Apparatus and Separation of DNA Fragments :-

1. Add loading dye to the DNA samples to be separated (Fig. 2). Gel loading dye is typically made at 6X concentration (0.25% bromphenol blue,

0.25% xylene cyanol, 30% glycerol). Loading dye helps to track how far your DNA sample has traveled, and also allows the sample to sink into the gel.

2. Program the power supply to desired voltage (1-5V/cm between electrodes).

3. Add enough running buffer to cover the surface of the gel. It is important to use the same running buffer as the one used to prepare the gel.

4. Attach the leads of the gel box to the power supply. Turn on the power supply and verify that both gel box and power supply are working.

5. Remove the lid. Slowly and carefully load the DNA sample(s) into the gel (Fig. 3). An appropriate DNA size marker should always be loaded along with experimental samples.

6. Replace the lid to the gel box. The cathode (black leads) should be closer the wells than the anode (red leads). Double check that the electrodes are plugged into the correct slots in the power supply.

7. Turn on the power. Run the gel until the dye has migrated to an appropriate distance.

3) Observing Separated DNA fragments :-

1. When electrophoresis has completed, turn off the power supply and remove the lid of the gel box.

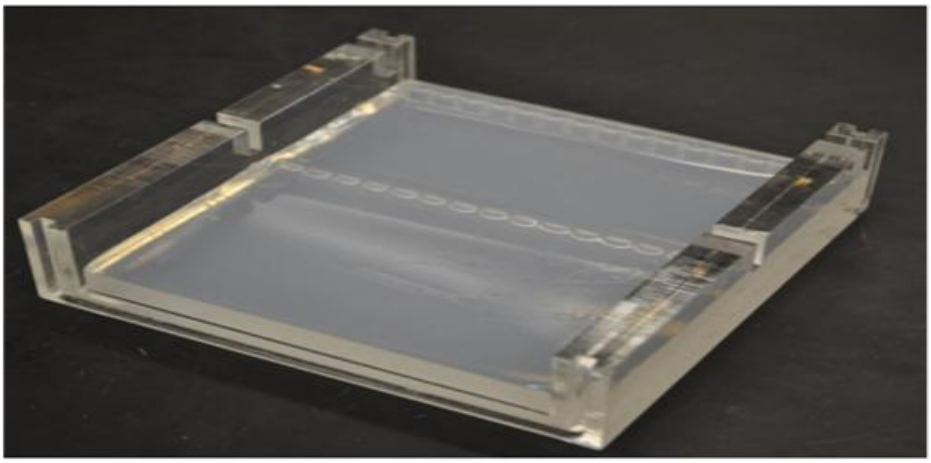
2. Remove gel from the gel box. Drain off excess buffer from the surface of the gel. Place the gel tray on paper towels to absorb any extra running buffer.

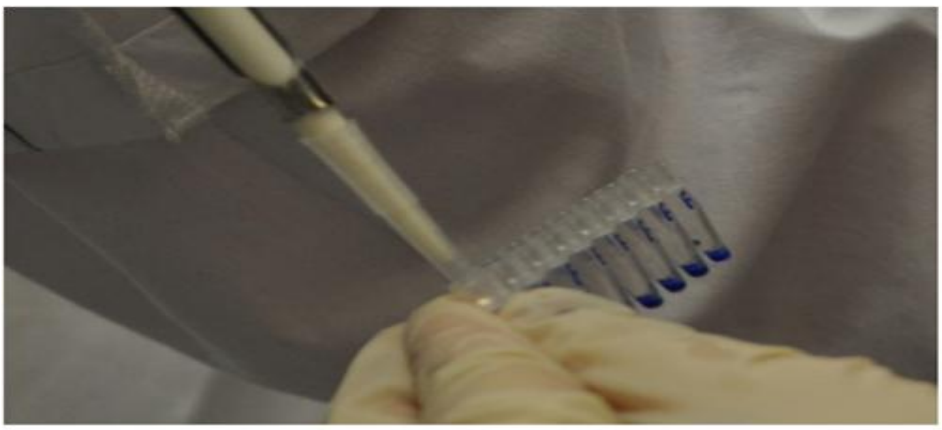
3. Remove the gel from the gel tray and expose the gel to uv light. This is most commonly done using a gel documentation system(fig.4) DNA bands should show up as orange fluorescent bands. Take a picture of the gel (fig.5)

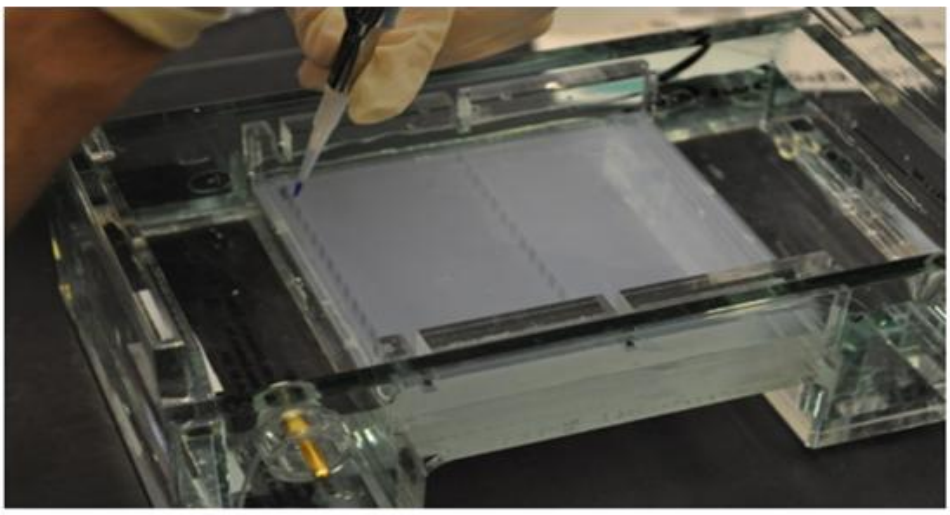
4. Properly dispose of the gel and running buffer per institution regulations.

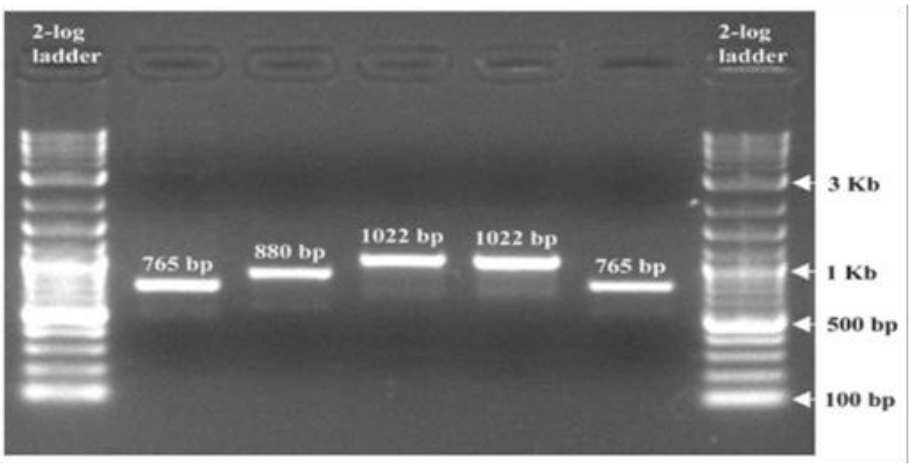
4) Representative Results :-

Represents a typical result after agarose gel electrophoresis of PCR products. After separation, the resulting DNA fragments are visible as clearly defined bands. The DNA standard or ladder should be separated to a degree that allows for the useful determination of the sizes of sample bands.









Apparent gel pore size

1) Agarose gels :-

Agarose is an alternating copolymer of 1,3-linked β-D-galactose and 1,4-linked 3,6- anhydro-α-L-galactose, infrequently substituted with carboxylate, pyruvate and/or sulfate residues . Agarose molecules in solution have a random coil structure at high temperatures. Upon cooling, the agarose chains form helical fiber bundles held together by noncovalent hydrogen bonds; gelation occurs at still lower temperatures when the fiber bundles become linked together in "junction zones" by the formation of additional hydrogen bonds . Strand partner exchange occurs in the junction zones by hydrogen bond rearrangements . The effective pore size of agarose gels can be estimated from Ferguson plots (log mobility vs. gel concentration of DNA molecules of different sizes.

Assuming a Gaussian distribution of pore sizes, the median pore radius of the gel in which the mobility of a given DNA molecule is reduced to one-half its mobility at zero gel concentration is equal to the radius of gyration of that DNA . Ferguson plots, such as those illustrated in were measured for normal DNA molecules ranging in size from 0.5 to 12.2 kilobase pairs in the first milestone paper cited above. The median pore radius of a 1% agarose gel was found to be ∼100 nm similar to the pore sizes obtained in other electrophoretic studies . However, the estimated gel pore radius depends somewhat on the method used to determine it.

2) Polyacrylamide gels

Polyacrylamide gels are chemically crosslinked gels formed by the reaction of acrylamide with a bifunctional crosslinking agent such as N,N′-methylenebisacrylamide (Bis). The composition of the gel is given by %T, the total (w/v) concentration of acrylamide plus crosslinker, and %C, the (w/w) percentage of crosslinker included in %T. Polyacrylamide gels are polydisperse in structure, because Bis polymerizes with itself more rapidly than with acrylamide . For this reason, polyacrylamide gels consist of highly crosslinked, Bis-rich nodules linked together by sparsely crosslinked, relatively acrylamide-rich fibers.

Because of the structural heterogeneity of the polyacrylamide gel matrix, the effective pore size determined by electrophoretic methods depends on the size of the analyte. If proteins are used as the analytes, the apparent pore size corresponds to the pores in the Bis-rich nodules Larger analytes, such as DNA, do not "see" the small pores in the nodules and instead are retarded by migration through the acrylamide-rich fibers In the second milestone paper cited above Ferguson plots were measured for DNA molecules ranging in size from 123 to 1600 bp in polyacrylamide gels containing 3.5% to 10.5%T and 3% Bis. Typical examples of the Ferguson plots obtained for multimers of 167-bp curved and normal DNA fragments are illustrated

Effect of the electric field on agarose and polyacrylamide gels :-

The mobilities observed for DNA molecules in agarose gels are highly dependent on the electric field applied to the gel most likely because the electric field disrupts the hydrogen bonds in the junctiones zones, allowing the gel fibers and fiber bundles to orient in the electric field . The oriented gel fibers and fiber bundles are very large, ranging up to 22 μm in length . Surprisingly, the gel fibers and fiber bundles orient in the perpendicular direction when the electric field is reversed in polarity . The resulting "flip-flop" orientation and reorientation of agarose fibers and fiber bundles in reversing electric fields provides a mechanism for creating transient pores in the gel matrix, allowing very large DNA molecules to migrate through the gel during pulsed field gel electrophoresis.

Polyacrylamide gels, which are chemically crosslinked, are distorted somewhat by the electric field, but significant orientation of the gel fibers does not occur . For this reason, DNA mobilities observed in polyacrylamide gels are essentially independent of the electric field strength used for electrophoresis.

DNA interactions with agarose and Polyacrylamide gel matricells

1)Agarose gels :-

According to the Ogston-Rodbard-Chrambach theory of gel electrophoresis the mobility of a polyelectrolyte in a gel matrix is determined by the fractional volume of the gel that is accessible to the migrating macromolecules. Hence, Ferguson plots are expected to extrapolate to the free solution mobility of an analyte at zero gel concentration. For DNA, the Ferguson plots should extrapolate to a common intercept at zero gel concentration, because the free solution mobilities of DNA molecules larger than ~400 bp are independent of molecular mass However, as shown for agarose gels in the intercepts at zero gel concentration decrease monotonically with increasing DNA size. Hence, DNA molecules migrating in agarose gels appear to be retarded by a molecular mass-dependent mechanism that occurs in addition to sieving. This molecular mass-dependent effect is most likely the transient interaction of the DNA molecules with the agarose gel fibers during electrophoresis.

2) Polyacrylamide gels

The Ferguson plots observed for DNA molecules in polyacrylamide gels extrapolate to very different mobilities at zero gel concentration If the mobilities observed at zero gel concentration are extrapolated linearly to zero DNA molecular mass, the free solution mobility of DNA is calculated to be (3.1 ± 0.1) × 10-4 cm2 / Vs in 40 mM Tris-acetate-EDTA buffer, equal within experimental error to the value obtained from extrapolation of the Ferguson plots observed in agarose gels. Hence, DNA molecules electrophoresed in polyacrylamide gels are also retarded by a molecular mass-dependent mechanism that occurs in addition to sieving, most likely transient interactions of the migrating DNA molecules with the gel matrix.

The relative importance of sieving and gel matrix interactions to the anomalously slow mobilities observed for curved DNA molecules in polyacrylamide gels can be evaluated by measuring the mobilities of curved and normal DNAs in gels in which the pore size is varied by changing %T at constant %C (the usual method of changing gel pore size) and by changing %C at constant %T. If sieving effects are the primary factor contributing to the anomalously slow mobilities, the mobility anomalies should be independent of the method used to vary the gel pore size.

Conclusion:-

The milestone papers cited in this review were the first of a series of papers designed to better understand why curved DNA molecules migrate anomalously slowly in polyacrylamide gels, but not in agarose gels.Thus, the electrophoresis of DNA fragments in agrose gels, polyacrylamide gels, free solutions is carried out essentially and clarified.

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