Study of different conformations of plasmid DNA by agarose gel electrophoresis

Theory

Agarose gel electrophoresis is a method used to separate DNA and RNA molecules according to their molecular size. This is achieved when negatively charged nucleic acids migrate through an agarose gel matrix under the influence of an electric field (electrophoresis). Shorter molecules with low molecular weight move faster and migrate farther than the larger ones. This method is simple, rapid to perform, and capable of resolving fragments of DNA that cannot be separated by other procedures such as density gradient centrifugation. The position of DNA in the agarose gel is visualized under UV light by staining with low concentration of fluorescent intercalating dyes, such as Ethidium bromide.

The following factors determine the rate of migration of DNA through agarose gels:

- Molecular size of DNA: Fragments of linear DNA migrate through agarose gel with a mobility that is inversely proportional to the log10 of their molecular weight. Larger molecules migrate more slowly because of greater frictional drag as they pass through the pores of the gel less efficiently than the smaller molecules. Circular forms of DNA migrate in agarose differently from linear DNA of the same mass. Undigested plasmids migrate more rapidly than the same plasmid when linearized.
- Agarose Concentration: By using gels with different concentrations of agarose, DNA fragments of different sizes can be resolved. Higher concentration of agarose facilitates separation of small DNA, while lower agarose concentration allows resolution of larger DNA. • Electrophoresis buffer: Several buffers have been recommended for electrophoresis of DNA. The most commonly used are TAE (Tris-acetate-EDTA) and TBE (Tris-borate-EDTA). DNA fragments will migrate at different rates in these two buffers due to difference in ionic strength. Buffers not only establish a pH, but also provide ions required to support conductivity.
- Effect of Ethidium bromide: Ethidium bromide is a fluorescent dye which fluoresces at 254–366nm. It intercalates between bases of nucleic acids and allows very convenient detection of DNA fragments in gels on a UV transilluminator. Binding of ethidium bromide to DNA alters its mass and rigidity, and therefore its mobility
- Voltage: As the voltage applied to a gel is increased, larger fragments migrate proportionally faster than smaller fragments. The best resolution of fragments larger than about 2 kb is attained byapplying voltage not more than 5 volts per cm to the gel (the cm value is the distance between the two electrodes, and not the length of the gel).

Materials Required

Plasmid DNA
Agarose
50XTAE buffer, control DNA
6X Gel Loading buffer

Ethidium bromide (10 mg/ml)

Procedure

1. Prepare gel tray by sealing the ends with adhesive tape. Place comb in the space provided on the gel tray.
2. To prepare 50 ml of 0.8 % agarose solution, add 0.4 g agarose to 50 ml 1X TAE buffer in a glass beaker or flask. Heat the mixture on a microwave/burner/hot plate by swirling the glass beaker/ flaskoccasionally, until agarose dissolves completely (Ensure that the lid of the flask is loose to avoid buildup of pressure).
3. Allow solution to cool down to about 55-60^{o} C. Add 0.5 µl Ethidium bromide, mix well and pour the gel solution into the gel tray. Allow the gel to solidify for about 30 minutes at room temperature.
4. To start the run, carefully remove the adhesive tape from both the ends of the gel tray, place the tray in electrophoresis chamber, and fill the chamber (just until wells are submerged) with 1X TAE electrophoresis buffer and gently remove the comb.
5. To prepare samples for electrophoresis, add 2 µl of 6X gel loading buffer for every 10 µl of DNA sample. Mix well and load the sample into the well. Load 3 µl of 1 Kb DNA Ladder into one of the well.
6. Connect the power cord to the electrophoretic power supply according to the conventions: RedAnode and Black- Cathode. Electrophorese at 100-120 volts and 90 mA until dye markers have migrated an appropriate distance, depending on the size of DNA to be visualized.
7. Electrophoresis apparatus should always be covered with the lid to avoid electric shocks. Avoid use of very high voltage as it can cause trailing and smearing of DNA bands in the gel, particularly with high molecular weight DNA. 8. Switch off the power supply once the tracking dye from the well reaches 3/4th of the gel which takes approximately 45 minutes.

Visualization and Interpretation of Agarose Gel

The illumination of a stained gel under UV light (254–366 nm) allows DNA bands to be visualized against a background of unbound dye. The gel image can be recorded by taking a Polaroid™ photograph or using a gel documentation system.

Plasmid DNA can exist in three conformations: supercoiled, open-circular (oc), and linear (supercoiled plasmid DNA is often referred to as covalently closed circular DNA, ccc). *In vivo*, plasmid DNA is a tightly supercoiled circle to enable it to fit inside the cell. In the laboratory, following a careful plasmid prep, most of the DNA will remain supercoiled, but a certain amount will sustain single-strand nicks. Given the presence of a break in only one of the strands, the DNA will remain circular, but the break will permit rotation around the phosphodiester backbone and the supercoils will be released. A small, compact supercoiled knot of ccc-DNA sustains less friction against the agarose matrix than does a large, floppy open circle of oc-DNA. Therefore, for the same over-all size, supercoiled DNA runs faster than open-circular DNA. Linear DNA runs through a gel end first and thus sustains less friction than open-circular DNA, but more than supercoiled. Thus, an uncut plasmid produces two bands on a gel, representing the oc and ccc conformations. If the plasmid is cut once with a restriction enzyme, however, the supercoiled and open-circular conformations are all reduced to a linear conformation. Following isolation, spontaneous nicks accumulate as a plasmid prep ages. This can clearly be seen on gels as the proportion of the two conformations change over time: plasmids preps that have been thawed and refrozen many times, show more oc DNA than fresh

preps

Quantitation of DNA

Spectrophotometric analysis and agarose gel electrophoresis will reveal the concentration and the purity of the genomic DNA. Use Elution Buffer to dilute samples and to calibrate the spectrophotometer, measure the absorbance at 260 nm, 280 nm, and 320 nm using a quartz microcuvette. Absorbance readings at 260 nm should fall between 0.1 and 1.0. The 320 nm absorbance is used to correct background absorbance. Purity is determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm. The concentration of DNA is calculated by the following formula: Concentration of DNA sample (µg/ml) = 50 x A260 x dilution factor

Ref. Calcutta University protocol