Lab : (2)
Level: (2)
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Gel Electrophoresis of DNA

This technique separates and purifies fragments of DNA or RNA as well as proteins. The basic idea of **electrophoresis** is to separate the molecules based on their electrical charge. Electrically positive charges attract negative charges and repel other positive charges. Conversely, negative charges attract positive charges and repel other negative charges. Two electrodes, one positive and the other negative, are connected up to a high voltage source. Positively charged molecules move towards the negative electrode and negatively charged molecules move towards the positive electrode. Since DNA carries a negative charge on each of the many phosphate groups making up its backbone, it will move towards the positive electrode during electrophoresis.

Most DNA is separated using **agarose gel electrophoresis**. **Agarose** is a polysaccharide extracted from seaweed. When agarose and water are mixed and boiled, the agarose melts into a homogeneous solution.

As the solution cools, it gels to form a meshwork, which has small pores or openings filled with water. The cooled gel looks much like a very concentrated mixture of gelatin without the food coloring. The pore size of agarose is suitable for separating nucleic acid polymers consisting of several hundred nucleotides or longer. Shorter fragments of DNA as well as proteins are usually separated on gels made of **polyacrylamide Gel (PAGE)**. The meshwork formed by this polymer has smaller pores than agarose polymers. PAGE is a powerful technique in the analysis of DNA molecules, and is able to very effectively separate DNA molecules that differ in size by as little as a single base pair. This high level of resolution makes PAGE ideal for the analysis of DNA sequence. The technique is,

however, limited to relatively small DNA molecules (less than 1000 bp in length). Large DNA molecules are unable to enter the pores of the polyacrylamide and are consequently not separated by the gel.

As the DNA molecules move through the gel they are hindered by the meshwork of fibers that make up the gel. The larger molecules find it more difficult to squeeze through the gaps but the smaller ones are slowed down much less. The result is that the DNA fragments separate in order of size , the rings of plasmid DNA will move farther in the gel than the chromosome.

THE RATE OF MIGRATION OF DNA THROUGH AGAROSE GELS

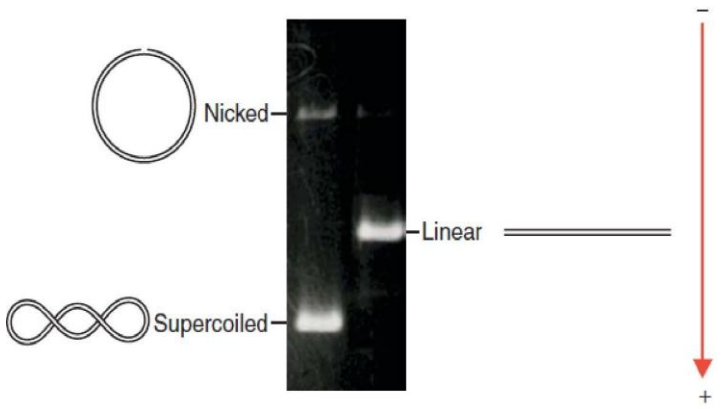
The following factors determine the rate of migration of DNA through agarose gels:

1. **The molecular size of the DNA**: Larger DNA molecules migrate more slowly than small molecules because of greater frictional drag.

2. **The concentration of agarose**: linear DNA fragments of a given size migrates at different rates through gels containing different concentration of agarose.

3. **The conformation of the DNA (topology**): Super helical circular, nicked circular, and linear DNA migrate through agarose gels at different rates. Plasmid DNA isolated from *E. coli* cells is invariably negatively supercoiled closed-circular molecules. These are relatively compact structures that run quickly through agarose gels. If one strand of the plasmid double helix becomes broken (nicked) then the supercoiling within the plasmid will be lost, and the more open structure of the relaxed plasmid will migrate more slowly through an agarose gel. If the same plasmid is treated with a restriction enzyme that cleaves it once, then this linearized DNA

will run with a mobility intermediate between those of the supercoiled and the nicked molecules. Therefore, DNA molecules that all contain precisely the same number of base pairs can run in several different locations on an agarose gel depending upon the topology of the DNA.



4. **The presence of ethidium bromide in the gel and electrophoresis buffer**: Intercalation of ethidium bromide causes a decrease in the negative charge of the double stranded and increase both its stiffness and length.

5. **The applied voltage**: The effective range of separation in agarose gels decreases as the voltages increased.

6. **The type of agarose**: The electrophoresis mobility of DNA is affected by the composition and ionic strength of the electrophoresis buffer, in the absence of ions electrical conductivity is minimal and DNA migrates slowly. In buffer of high ionic strength electrical conductance is very efficient and significant amounts of heat are generated and the gel melts and the DNA denatures.

The required equipment for conducting agarose gel electrophoresis:

1. Electrophoresis chamber.
2. Power supply.
3. Gel casting trays.
4. Combs.
5. Trans illuminator (an ultraviolet lightbox).

Required buffers and dye

1. **Electrophoresis buffer (10x)**, usually Tris-acetate-EDTA (TAE) or Tris-borate-EDTA (TBE).
2. **Loading buffer (6x)**, which contains something dense (e.g. glycerol) to allow the sample to "fall" into the sample wells, and one or two tracking dyes, which migrate in the gel and allow visual monitoring or how far the electrophoresis has proceeded.
3. **Ethidium bromide (10 mg/ml in final con. 0.5 µg/ml)**, a fluorescent dye used for staining nucleic acids is able to intercalate between the stacked base pairs of DNA, Ethidium bromide will bind very efficiently to double-stranded DNA, but less so to single-stranded DNA and RNA because of the relative lack of base stacking.
4. Agarose gel 0.7-1% in 1X TBE**.**

Casting the Agarose Gel

1. Close off the open ends of a clean and dry gel bed (casting tray) by using rubber dams or tape.
2. Place a well-former template (comb) in the first set of notches at the end of the bed. Make sure the comb sits firmly and evenly across the bed.

3. Use a 250mL flask to prepare the gel solution. Add the following components to the flask.
    a. 0.24 g of Agarose
    b. 0.6 mL of concentrated buffer solution
    c. 29.4 mL of distilled water

4. Swirl the mixture to dispense clumps of agarose powder
5. With a marking pen, indicate the level of the solution volume on the outside of the flask.
6. Heat the mixture to dissolve the agarose powder. The final solution should appear clear (like water) without any dissolved particles
7. Cool the agarose solution to 55oC with careful swirling to promote even dissipation of heat. If detectable evaporation has occurred, add distilled water to bring the solution up to the original volume as marked on the flask previously.
8. Pour the cooled agarose solution into the bed. Make sure the bed is on a level surface.
9. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes.

Preparing the Chamber

1. After the gel is completely solidified, carefully and slowly remove the rubber dams.
2. Remove the comb slowly by pulling straight up. Do this carefully and evenly to prevent tearing the sample wells.
3. Place the gel (on its bed) into the electrophoresis chamber, properly oriented, centered and level on the platform.
4. Fill the electrophoresis apparatus chamber with the required volume of diluted buffer for the specific unit you are using.
5. Make sure the gel is completely covered with buffer.
6. Proceed to loading the samples and conducting electrophoresis.

Loading Samples:

1. The amount of sample that should be loaded is 35-38 microliters.

Running Samples:

1. After the samples are loaded, carefully snap the cover down onto the electrode terminals. Make sure that the negative and positive color-coded indicators on the cover and apparatus chamber are properly oriented.

2. Insert the plug of the black wire into the black input of the power source (negative input). Insert the plug of the red wire into the red input of the power source (positive input).
3. Set the power source at the required voltage and conduct electrophoresis for the length of time determined by your instructor.
4. Check to see that current is flowing properly - you should see bubbles forming on the two platinum electrodes.
5. After approximately 10 minutes, you will begin to see separation of the colored dyes.
6. After the electrophoresis is completed, turn off the power, unplug the power source, disconnect the leads and remove the cover.
7. Document the gel results.

If you see faint or no bands on the gel:

- There was insufficient quantity or concentration of DNA loaded on the gel.
- The DNA was degraded.
- The DNA was electrophoresed off the gel.
- Improper W light source was used for visualization of ethidium bromide-stained DNA.

- **If you see smeared DNA bands:**

- The DNA was degraded. Avoid nuclease contamination.
- Too much DNA was loaded on the gel. Decrease the amount of DNA.
- Improper electrophoresis conditions were used.
- The DNA was contaminated with protein.

If you see anomalies DNA band migration:

- Improper electrophoresis conditions were used. Do not allow voltage to exceed ~20 V/cm. Maintain a temperature <30° C during electrophoresis. Check that the electrophoresis buffer used had sufficient buffer capacity.
- The DNA was denatured.