Genetic engineering
Department of Biology
College of Science
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Lab: 3

Nucleic acid (DNA/RNA) Qualitative analysis and Quantitative analysis

Gel Electrophoresis for DNA/ RNA analysis (Qualitative analysis Size-Quality

Gel electrophoresis is the standard lab procedure for separating DNA by size (e.g. length in base pairs) for visualization and purification. Electrophoresis uses an electrical field to move the negatively charged DNA toward a positive electrode through an agarose gel matrix. The gel matrix allows shorter DNA fragments to migrate more quickly than larger ones. Thus, you can accurately determine the length of a DNA segment by running it on an agarose gel alongside a DNA ladder (a collection of DNA fragments of known lengths).

The materials and components are using in electrophoresis:.

1- Buffer TBE (**Tris/Borate/EDTA**) or TAE (**Tris-acetate-EDTA**) **(or TPE Tris-Phosphate EDTA)**.
2- Agarose
3- Loading dye buffer (6X): Bromophenol blue or orange G + glycerol and/ or xylene cyanol FF

4- Fluorescent dye: Ethidium bromide or gel red, gel green or Sybr safe.
5-Power supply
6-Cell
7-Tray or gel bed
8-Comb
9- Ultraviolet cabinet
Several additional factors have important effects on mobility of DNA fragments in agarose gels:

1. Agarose concentration, 1% gels are common for many applications. (Agarose gel electrophoresis can be used for the separation of DNA fragments ranging from 50 base pair to several megabases (millions of bases) using specialized apparatus. Increasing the agarose concentration of a gel reduces the migration speed and enables separation of smaller DNA molecules).

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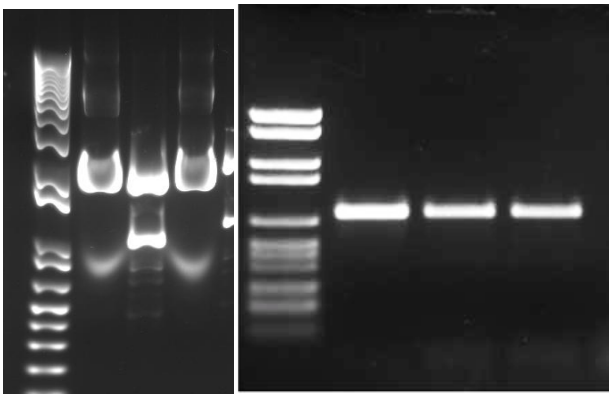
2. Voltage (The higher the voltage, the faster the DNA moves. But voltage is limited by the fact that it heats and ultimately causes the gel to melt
   High voltages also decrease the resolution (above about 5 to 8 V/cm).



The effect of using high voltage.

3. **Electrophoresis Buffer**
   The two most popular types of buffers for running agarose gels are Tris-acetate with EDTA (TAE) and Tris-borate with EDTA (TBE). Because both buffers have a near-neutral pH, the DNA in the buffers has a net negative charge and migrates toward the anode (+) end of the gel apparatus.

4. **Effects of Ethidium Bromide.**

Procedur :

1- Prepare 1% agarose gel by adding 1 gm of agarose to 100 ml of 1x TBE buffer, heat until the agarose completely dissolved then allow the gel to cool to 45-50 °C.
2- The next step, adds 5µl of Ethidium bromide (final concentration 0.5 µg/mL), mix well and pour carefully in the tray (always avoid air bubbles formation between the teeth of the comb). Allow the gel to set at room temperature for 30-40 minutes.
3- Remove the comb carefully from the gel and place the tray in the gel tank, after that add a sufficient 1xTBE buffer to the tray (should cover the gel).
4- The gel is ready to load samples (e.g amplified PCR products) and run your gel for 1 hour and a half (7 Volts/ cm).
5- DNA ladder is used to determine samples size (e.g PCR product size), and then PCR products are visualized by UV light at 336 nm, and are photographs by using digital camera.

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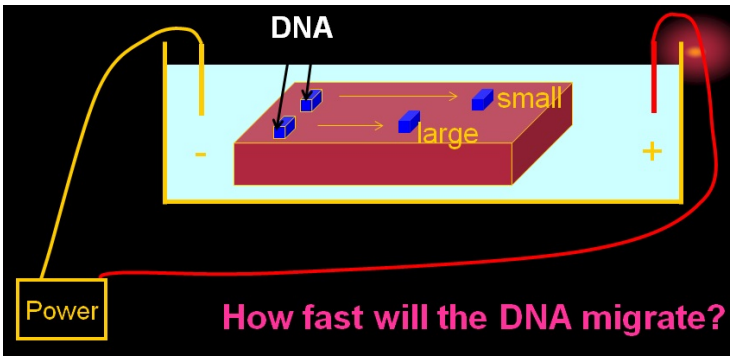


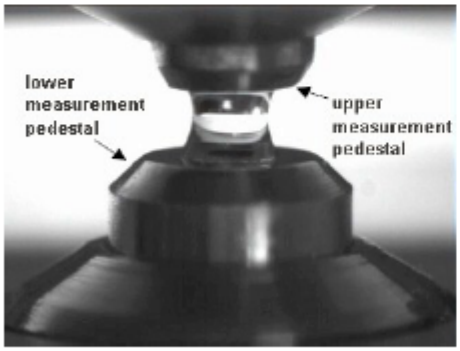
Nanodrop Spectrophotometry (Quantitative analysis, Purity- Yield)

Nanodrop

The rings of the bases (A, C, G, T, U) are made up of alternating single and double bonds. Such ring structures absorb in the U.V. Each of the four-nucleotide bases has a slightly different absorption spectrum, and the spectrum of DNA is the average of them.

The main steps for using the sample retention system are listed below:

1. With the sampling arm open, pipette the sample onto the lower measurement pedestal.



2. Close the sampling arm and initiate a spectral measurement using the operating software on the PC. The sample column is automatically drawn between the upper and lower measurement pedestals and the spectral measurement made.

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