Genetic engineering practical class

Lab 3

Gel Electrophoresis

Principle of gel electrophoresis

It is one of the most technique method that is used in biochemistry & molecular biologyto separate and analysis macromolecules (such as: DNA, RNA & proteins) and
their fragments, based on their mass, size and charge.

When these molecules are placed in matrix of gel and an electrical current is applied, they will move through the pores of the gel towards either the anode or cathode according to the net charge of molecules.

Migration: The movement of charged molecules through the pores of the gel from one electrode to another.

Sieving: Smaller molecules are considered less impeded by the gel matrix and hence will move faster than larger molecules.

Ex.:

1. DNA and RNA are negatively charged molecules due to the presence of a phosphate group (PO43–) on each nucleotide; they will be moved toward the anode (positively pole) of the gel.

2. Proteins, may be folded into a variety of shapes (affecting size) and have positive and negative regions (no clear charge), for that, it must first be treated with an anionic detergent such as sodium dodecyl sulfate (SDS) in order to linearized and coats them with a negative charge, which allows them to migrate toward the anode of the gel .

   Finally, after the DNA, RNA, or protein molecules have been separated by using gelelectrophoresis, bands representing molecules of different sizes can be detected.

Material of gel electrophoresis:

There are several materials that must be used to accomplished an electrophoresis process, such as:

Gel:

It the matrix that molecules are migrated through. There are different types of gel used according to the mixture of molecules that want be to separate depending on pore size of the gel, such as agarose and polyacrylamide (which is used for proteins electrophoresis).

Types of Gel:

1)**Agarose:** It is a polysaccharide extracted from seaweed, which comes as dry, powdered flakes. It is easy to prepare by using electrophoresis buffers and typically used at concentration of 0.5 - 3% for sieving migrated DNA & RNA. This gel is a matrix of agarose molecules that are held together by hydrogen bonds and form tiny pores.

2) **Polyacrylamide:** Polyacrylamide gel electrophoresis (PAGE) is used for separating proteins ranging in size from 5 to 2,000 kDa due to the uniform pore size provided by the polyacrylamide gel. Pore size is controlled by modulating the concentrations of acrylamide and bis-acrylamide powder used in creating a gel .The monomers react with each other by cross-linked. The length of the polymer chains is dictated by the concentration of acrylamide used, which is typically between 3- 30% .It prepared by mixed acrylamide and bis-acrylamide powder in electrophoresis buffer. Actually, sometime adding free radicals when associated with water to activate polymerization and must avoiding air bubbles by vacuum because they prevent forming of free radicals and polymerization.

Ex: The following solutions used to preparation of SDS-PAGE.

1-(Resolving gel) Separation gel 12%(1mm thickness):

It was prepared from acryl amide bis acryl amide, Tris-HCl pH=8.8, ammonium persulphate (APS) , Glycerol, and TEMED. The ingredients were mixed well in Distilled water and poured into glass plate sandwich for electrophoresis and n-butyl alcohol or distilled water was added on the surface of mixture and left to polymerize for at least 30-45 minutes.

Stacking gel % 4 (1 mm thickness): it was prepared from same separation gel but with different concentration. it prepared from acryl amide bis -acrylamide, Tris-HCl pH=6.8, ammonium persulphate (APS), TEMED, The n-butyl alcohol or distilled water was removed from the surface of separating gel; the comb was fixed well then the stacking gel was pipetted on the separation gel until the sandwich space was filled, the gel was left to polymerize for at least10-20 minute.

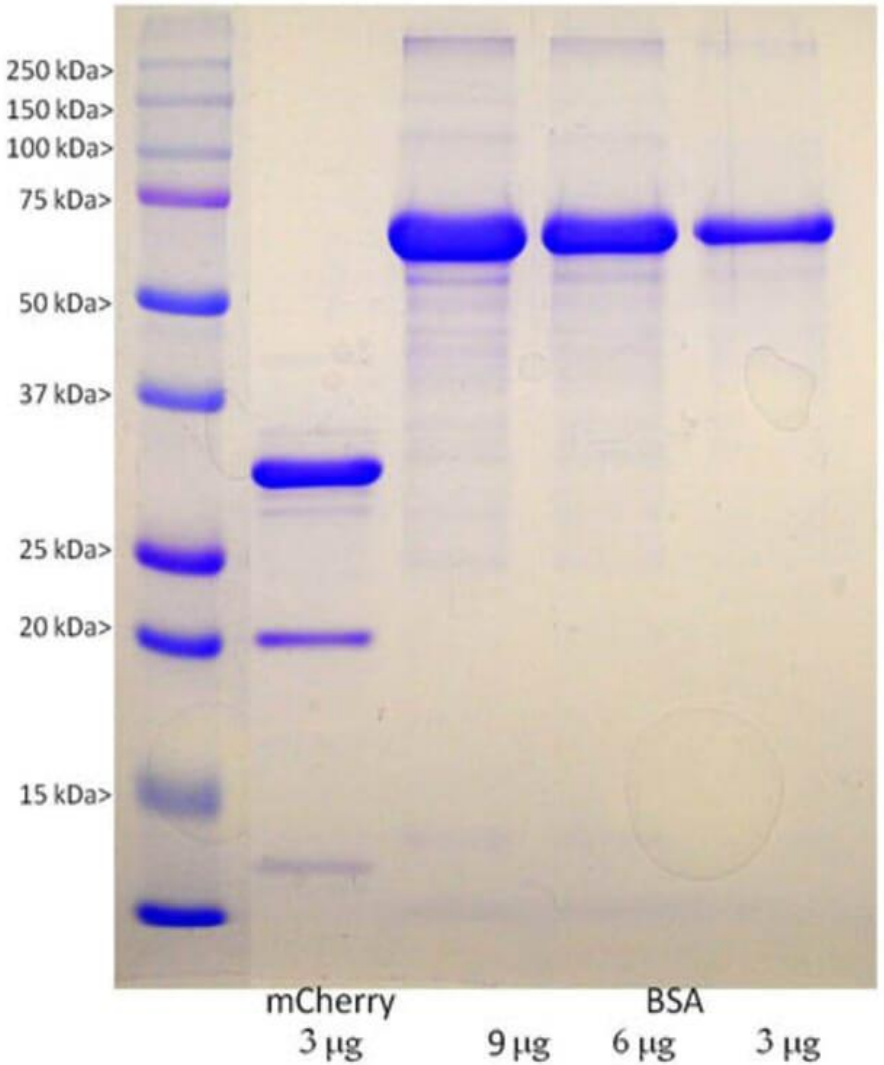
https://www.youtube.com/watch?v=EDi_n_0NiF4

Electrophoresis Buffers:

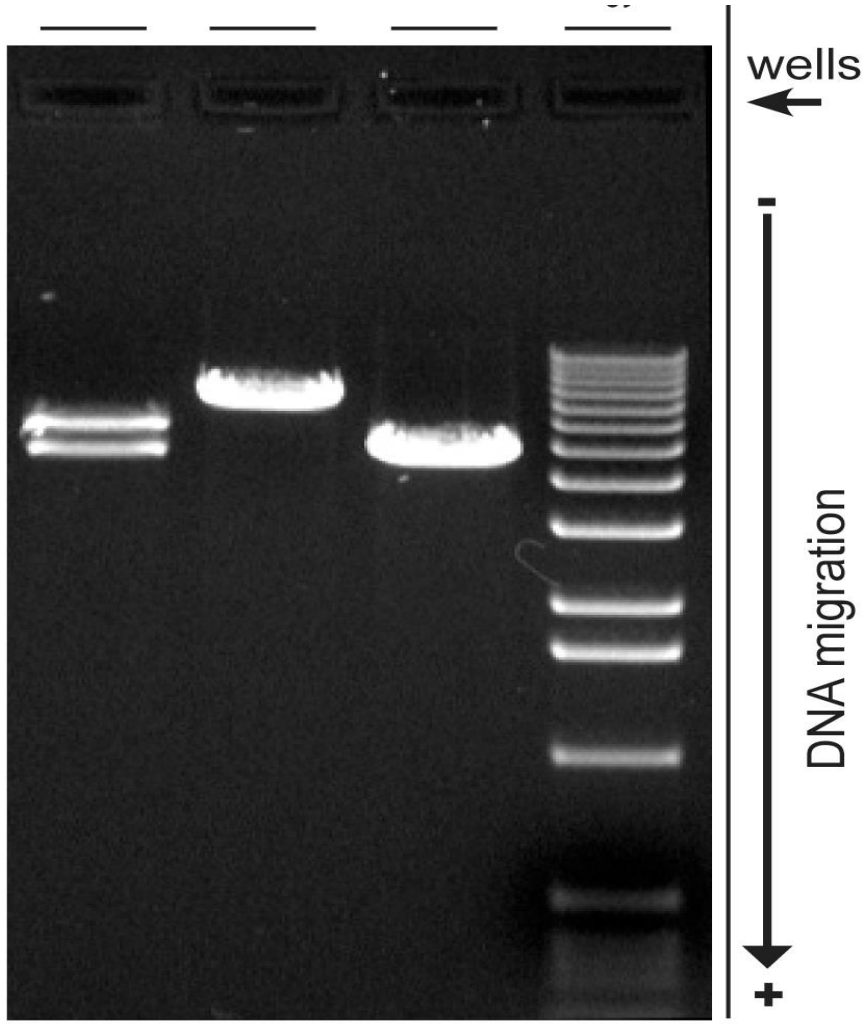
Buffers in gel electrophoresis are used to provide ions that carry a current and to maintain the pH at a relatively constant value. These buffers have plenty of ions in them, which is necessary for the passage of electricity through them. These buffers are preparing as 10X and diluted to 1X for preparing gel and electrophoresis process.

The composition & ionic strength of the electrophoresis buffer affects the electrophoretic mobility of DNA. This is important because the structure and charge of a protein or nucleic acid will change if pH changes, thus preventing proper separation. Therefore, solutions such a: distilled water or benzene that contains few are not ideal for the use in electrophoresis because it make molecules migrates slowly.

SDS gel for protein



Agaros gel for DNA, and RNA



There are many buffers used for electrophoresis such as:

1 Tris acetate EDTA (TAE)

2 Tris borate EDTA (TBE)

3) Loading Buffers:

This buffer is consisting of several martials, as the following:

1)Stop mix:

one has specific role in this process, it is a compound, which may be glycerol, sucrose, or Ficoll. These compounds increase the weight of DNA sample and prevent its floating because of the low density of DNA. If the DNA sample contains residual ethanol after its preparation, it may float out of the well.

2)Tracking dye:

used as indicator of DNA sample migration, and it is allowing visual mentoring the molecule in the gel; such as: bromophenol blue and xylene cyanol

❖ To preparation of loading buffer, take 25 mg bromophenol blue & 4 gm sucrose and mixed with 10 ml of D.W, then stored at 4C.

https://www.youtube.com/watch?v=HncilgQlzKE

4) Staining and visualization

1. For DNA and RNA

they are staining by ethidium bromide, which intercalates into the major grooves of the DNA and fluoresces under UV light. The intercalation depends on the concentration of DNA and thus, a band with high intensity will indicate a higher amount of DNA compared to a band of less intensity. However, this dye, it is a known mutagen material and should be handle as a hazardous chemical, which required wearing gloves during handling.

2. For protein:

the Staining of gel by Coomassie brilliant blue R-250 for 4 hours with shaking, but to clearly the proteins band need to remove the excise dye by De- staining solution that consist acetic acid and 95% methanol .