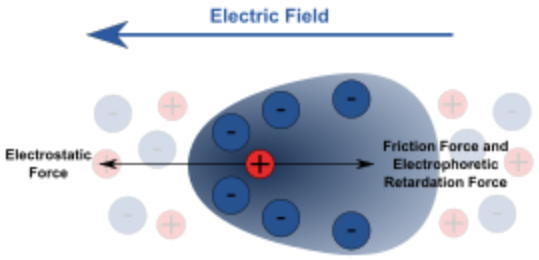
Electrophoresis

What is electrophoresis?

Electrophoresis is an electrokinetic process which separates charged particles in a fluid using a field of electrical charge. It is most often used in life sciences to separate protein molecules or DNA and can be achieved through several different procedures depending on the type and size of the molecules. The procedures differ in some ways but all need a source for the electrical charge, a support medium and a buffer solution. Electrophoresis is used in laboratories for the separation of molecules based on size, density and purity.



The procedures differ in some ways but all need a source for the electrical charge, a support medium and a buffer solution. Electrophoresis is used in laboratories for the separation of molecules based on size, density and purity.

How does it work?

An electric field is applied to molecules and as they are electrically charged themselves it results in a force acting upon them. The greater the charge of the molecule the greater the force applied by the electrical field and therefore the further through the support medium the molecule will move relative to its mass.

Some example applications of electrophoresis include DNA and RNA analysis as well as protein electrophoresis which is a medical procedure used to analyse and separate the molecules found in a fluid sample (most commonly blood and urine samples).

Types of gel used in electrophoresis

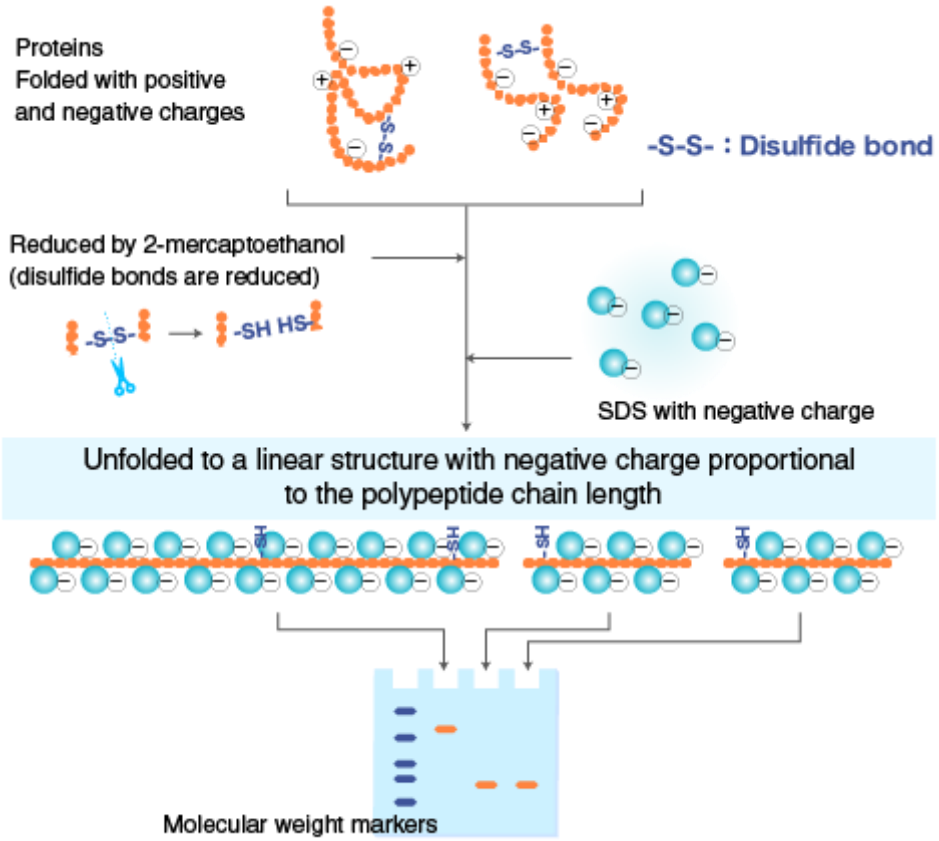
- Different types of gels are usually used as the support medium for electrophoresis and this may be in slab or tube form depending on which is more beneficial. Gel slabs enable many samples to be run simultaneously and so are frequently used in laboratories. However, tube gels give a better resolution of the results so are often chosen for protein electrophoresis.

- Agarose gel is commonly used for electrophoresis of DNA. It has a large pore structure allowing larger molecules to move easily but it is not suitable for sequencing smaller molecules.
- Polyacrylamide gel electrophoresis (PAGE) has a clearer resolution than agarose gel making it more suitable for quantitative analysis. This makes it possible to identify how proteins bind to DNA. It can also be used to develop the understanding of how bacteria is becoming resistant to antibiotics through plasmid analysis.
- 2D Electrophoresis separates molecules along an x-axis and a y-axis – one separating them by charge and the other by size.

The principle and method of polyacrylamide gel electrophoresis (SDS-PAGE)

- SDS-PAGE is an analytical technique to separate proteins based on their molecular weight.

The principle

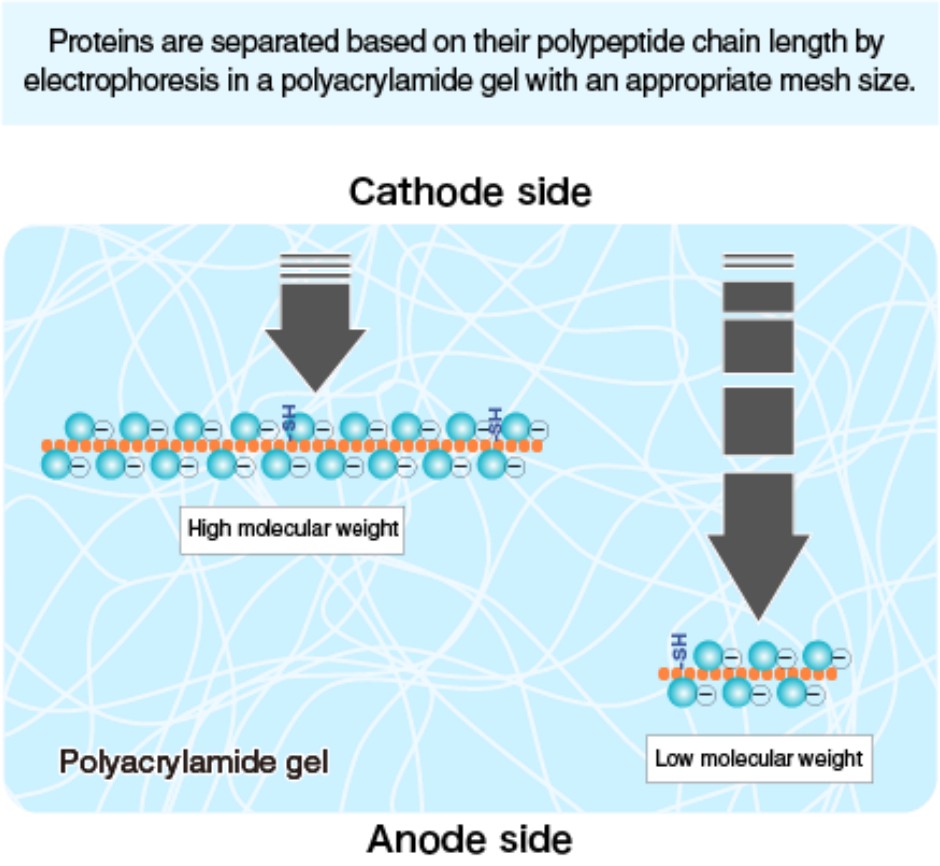


When proteins are separated by electrophoresis through a gel matrix, smaller proteins migrate faster due to less resistance from the gel matrix. Other influences on the rate of migration through the gel matrix include the structure and charge of the proteins.

In SDS-PAGE, the use of sodium dodecyl sulfate (SDS, also known as sodium lauryl sulfate) and polyacrylamide gel largely eliminates the influence of the structure and charge, and proteins are separated solely based on polypeptide chain length.

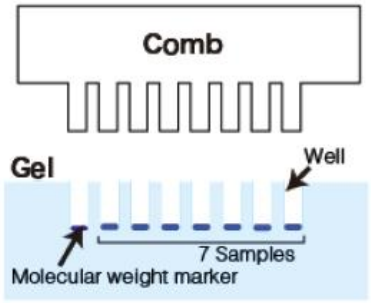
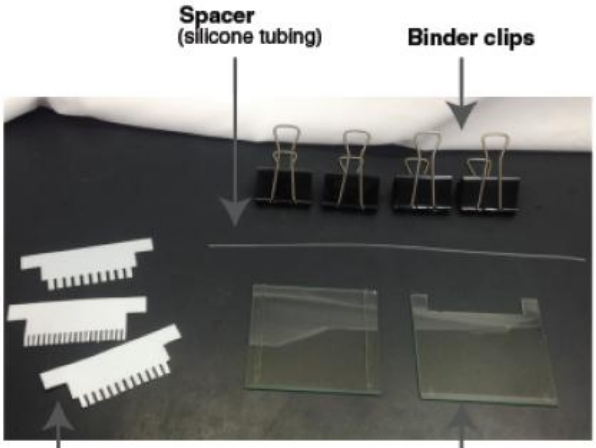
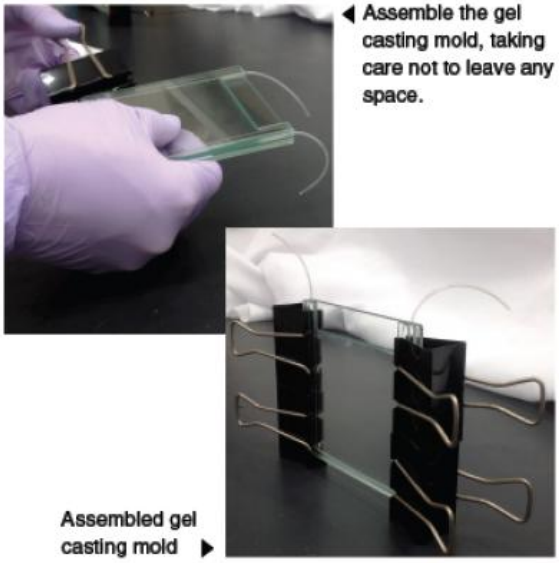
SDS is a detergent with a strong protein-denaturing effect and binds to the protein backbone at a constant molar ratio. In the presence of SDS and a reducing agent that cleaves disulfide bonds critical for proper folding, proteins unfold into linear chains with negative charge proportional to the polypeptide chain length.

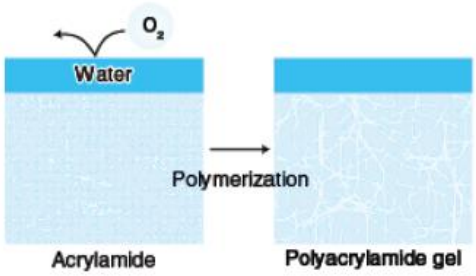
Polymerized acrylamide (polyacrylamide) forms a mesh-like matrix suitable for the separation of proteins of typical size. The strength of the gel allows easy handling. Polyacrylamide gel electrophoresis of SDS-treated proteins allows researchers to separate proteins based on their length in an easy, inexpensive, and relatively accurate manner.



Procedure

Preparation of polyacrylamide gel ※An example performed at MBL

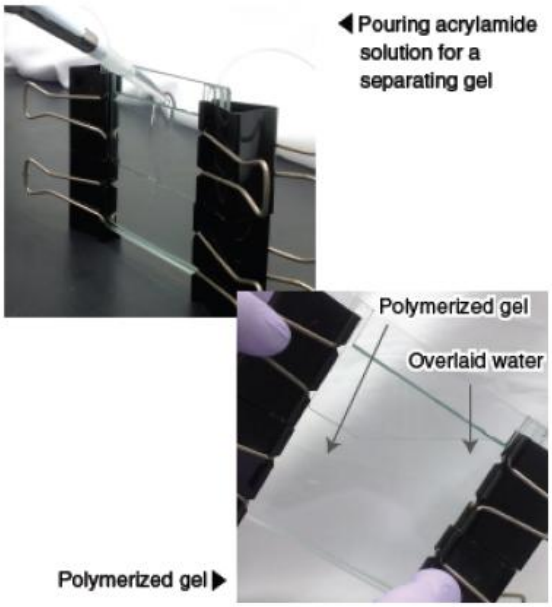
Step-by-step procedure	
Gather combs, glass plates, spacer (silicone tubing), and binder clips. A comb is used to make wells (lanes) to load samples. Use an appropriate comb depending on the sample size. Example: Use an 8-lane comb for 7 samples and molecular weight markers. 	
Thoroughly clean the glass plates with ethanol, and assemble the gel casting mold.	
Pour acrylamide solution for a separating gel. Overlay with water to prevent contact with air (oxygen), which inhibits polymerization. Allow acrylamide to polymerize for 20-30 minutes to form a gel. Remove the overlaid water.	



Proteins migrate at different rate depending on the concentration of the separating gel. Use an appropriate gel concentration for your target protein.

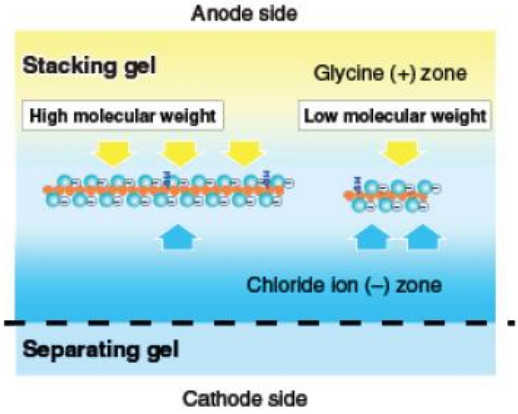
Using a higher acrylamide concentration produces a gel with a smaller mesh size suitable for the separation of small proteins. In general, an acrylamide concentration between 6 and 15% is used.

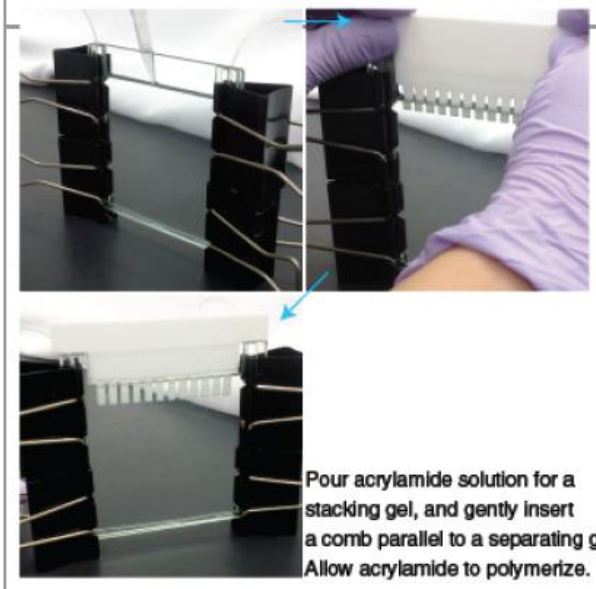
Gels with an acrylamide concentration gradient (gradient gels) are also used.



Pour acrylamide solution for a stacking gel; insert a comb and allow the acrylamide to polymerize.

Proteins are highly concentrated when they migrate through a stacking gel prior to entering a separating gel. The concentration occurs due to the difference in the rate of migration of glycine ion, chloride ion, and proteins, as illustrated below.



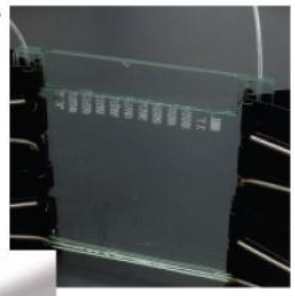
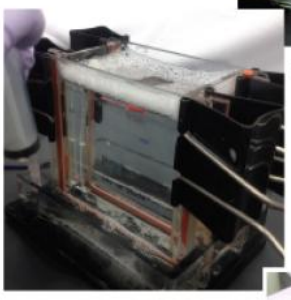
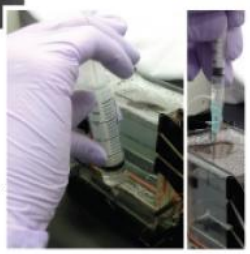
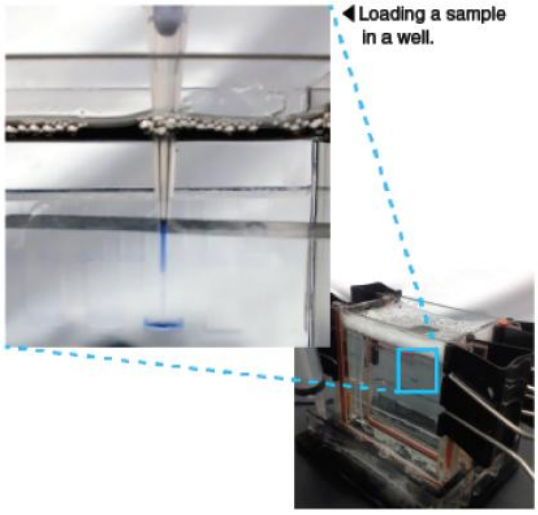
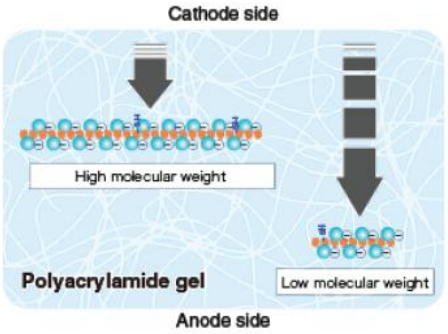
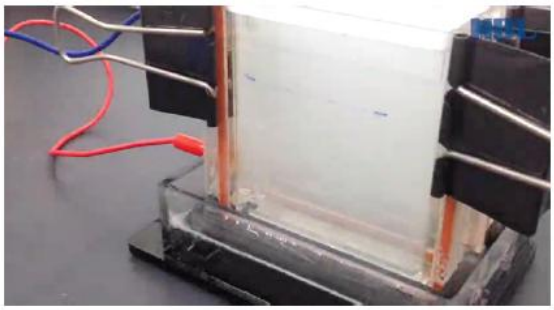


Pour acrylamide solution for a stacking gel, and gently insert a comb parallel to a separating gel. Allow acrylamide to polymerize.

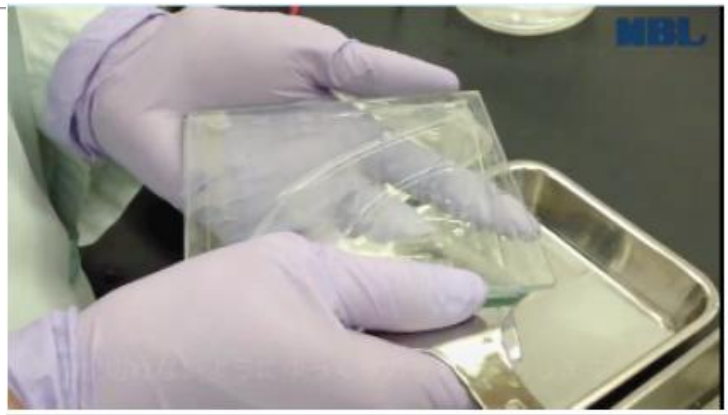
Preparation of samples ※An example performed at MBL

Step-by-step procedure	
Add sample buffer to samples, and mix by flicking the tube. **Heat the samples at 100°C for 3 minutes in a heat block.** **Centrifuge at 15,000 rpm for 1 minute at 4°C, and use the supernatant for SDS-PAGE.**	 ◀Add sample buffer to the samples and heat.

Electrophoresis ※An example performed at MBL

Step-by-step procedure	
Remove the binder clips, spacer, and comb from the gel assembly, and mount the gel in the electrophoresis apparatus using binder clips. **Pour running buffer into the upper and lower chambers of the electrophoresis apparatus, and remove air bubbles and small pieces of gel from the wells and under the gel using a syringe.**	The comb is removed from the gel assembly. ▶ Remove the spacer and binder clips.   ◀ The gel is mounted on the electrophoresis apparatus, and running buffer is poured.  Remove air bubbles and small pieces of gel using a syringe. ▶
Load samples and molecular weight markers in wells.	 ◀ Loading a sample in a well.
Turn on the power supply, and run the gel until the dye (BPB) in the sample buffer reaches the bottom of the gel.  	

Remove the gel assembly from the electrophoresis apparatus. Remove the gel from the glass plates using a spatula, and prepare for subsequent analysis.



Link for further studies: https://ruo.mbl.co.jp/bio/e/support/method/sds-page.html

APPLICATIONS OF SDS-PAGE

SDS-PAGE is used mainly for the following purposes:

1. Measuring molecular weight.
2. Peptide mapping.
3. Estimation of protein size.
4. Determination of protein subunits or aggregation structures.
5. Estimation of protein purity.
6. Protein quantitation.
7. Monitoring protein integrity.
8. Comparison of the polypeptide composition of different samples.
9. Analysis of the number and size of polypeptide subunits.
10. Post-electrophoresis applications, such as Western blotting.
11. Staining of Proteins in Gels with Coomassie G-250 without Organic Solvent and Acetic Acid.
12. Pouring and Running a Protein Gel by reusing Commercial Cassettes.
13. Selective Labelling of Cell-surface Proteins using CyDye DIGE Fluor Minimal Dyes.
14. Detection of Protein Ubiquitination.
15. SDS-PAGE/Immunoblot Detection of Aβ Multimers in Human Cortical Tissue Homogenates using Antigen-Epitope Retrieval.

ADVANTAGES & DISADVANTAGES

- **Advantages:**
  1. Migration of the molecules is proportional to their molecular weights.
  2. Highly sensitive test, separates molecules that have even a 2% difference in mass.
  3. Requires very small amounts of samples.
  4. A stable chemically cross-linked gel is used.
- **Disadvantages:**
  1. Poor band resolution due to high alkaline operating pH.
  2. Acrylamide gel is a potent neurotoxin chemical.
  3. Gel preparation is difficult and takes a long time.
  4. Very costly.

Differences

Native PAGE

- Separation is based upon charge, size, and shape of macromolecules.
- Useful for separation and/or purification of mixture of proteins
- This was the original mode of electrophoresis.

SDS PAGE

- Separation is based upon the molecular weight of proteins.
- The most common method for determining MW of proteins
- Very useful for checking purity of protein samples

Native Gel Electrophoresis

Clip slide

Native PAGE

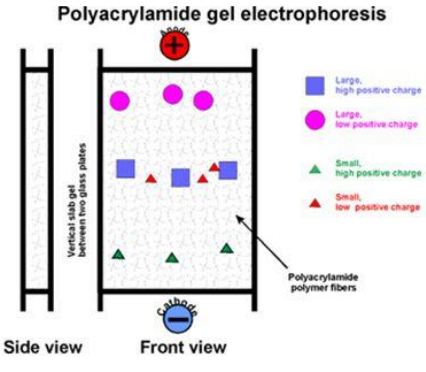
- Sometime, we need to **separate protein in non-denaturing conditions**. This type of polyacrylamide gel electrophoresis is also called native gel electrophoresis because protein remains in native form even after electrophoresis

Difference between Native & SDS PAGE

- The basic difference in the native gel electrophoresis (native-PAGE) is the electrophoresis buffer does not contain SDS.
- Also, loading buffer does not have SDS and reducing agents and samples are not boiled.
- Rest of the things are similar to SDS- PAGE gel electrophoresis

Native Gel Conditions

- Use PA support
- No Denaturant
  - Protein stays in original conformation
  - Protect from Oxidation
- Movement depends on:
  - Intrinsic Charge[7]
  - Hydrodynamic Size



http://ccnmtl.columbia.edu/projects/biology/lecture6/index.htm

Advantages of native PAGE

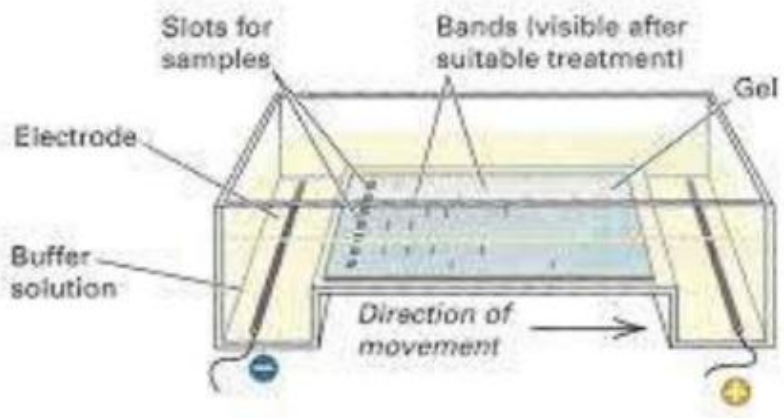
- In conjugation with SDS-PAGE,horizontal PAGE used to seprate & analyze complex biological sample
- User friendly & no specialized equipment required
- Native preparative gel to purify proteins in bulk for activity assay,antibody development etc

- Biological activity of protein remain intact
- Native PAGE is used for separation of enzymes/isozymes.

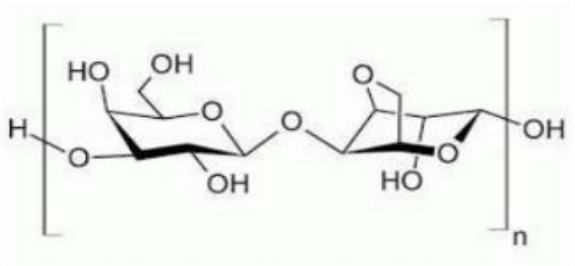
Agarose Gel Electrophoresis

ELECTROPHORESIS

Agarose gel electrophoresis of DNA



Agarose gel electrophoresis is the easiest and most popular way of separating and analyzing DNA. Here DNA molecules are separated on the basis of charge by applying an electric field to the electrophoretic apparatus. Shorter molecules migrate more easily and move faster than longermolecules through the pores of the gel and this process is called sieving. The gel might be used to look at the DNA in order to quantify it or to isolate a particular band. The DNA can be visualized in the gel by the addition of ethidium bromide.



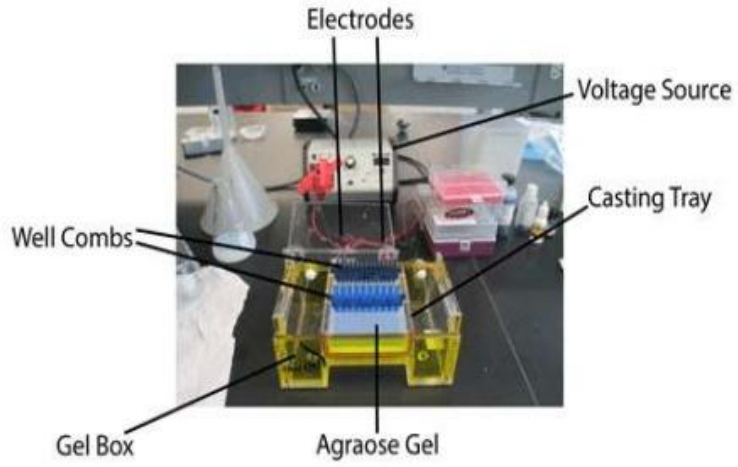
Agarose is a polysaccharide obtained from the red algae Porphyra umbilicalis. Its systematic name is (1 4)-3, 6-anhydro-a-L-galactopyranosyl-(1,3)-β-D-galactopyranan. Agarose makes an inert matrix. Most agarose gels are made between 0.7% and 2% of agarose. A 0.7% gel will show good separation for large DNA fragments (5-10kb) and a 2% gel will show good resolution for small fragments with size range of 0.2-1kb.

Materials Required

- Gel casting trays, which are available in a variety of sizes and composed of Uv transparent plastic.
- Sample combs, around which molten agarose is poured to form sample wells in the gel.
- Electrophoresis buffer, usually Tris-acetate-EDTA (TAE) or Tris-borate-EDTA (TBE).

The migration rate of DNA fragments in both of these buffers is somewhat different due to the differences in ionic strength. These buffers provide the ions for supporting conductivity.

- Loading buffer, 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.



Ethidium Bromide (EtBr)

Ethidium bromide, a fluorescent dye used for staining nucleic acids. It is an intercalating agent which intercalates between nucleic acid bases and allows the convenient detection of DNA fragments in gel. When exposed to UV light, it will fluoresce with an orange colour. After the running of DNA through an EtBr-treated gel, any band containing more than ~20 ng DNA becomes distinctly visible under UV light. EtBr is a known "mutagen", however, safer alternatives are available. It can be incorporated with agarose gels or DNA samples before loading, for visualization of the fragments. Binding of Ethidium bromide to DNA alters its mass and rigidity, and thereby its mobility.

Transilluminator (an ultraviolet light box), which is used to visualize ethidium bromide stained DNA in gels.

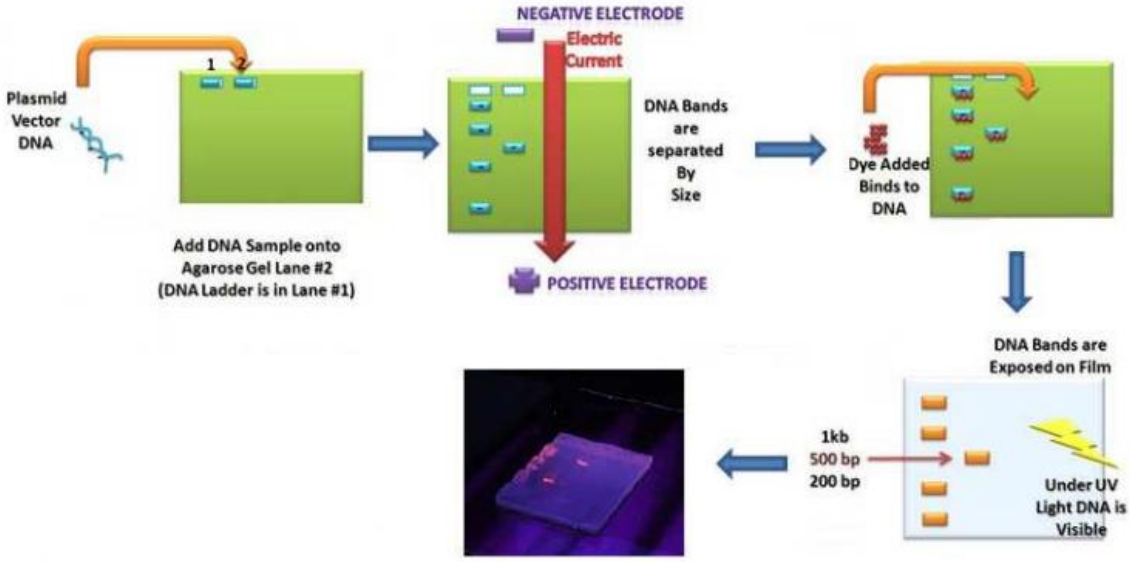


Fig. 1. Agarose gel electrophoresis method (modified from http://www.molecularstation.com/agarose-gel-electrophoresis).

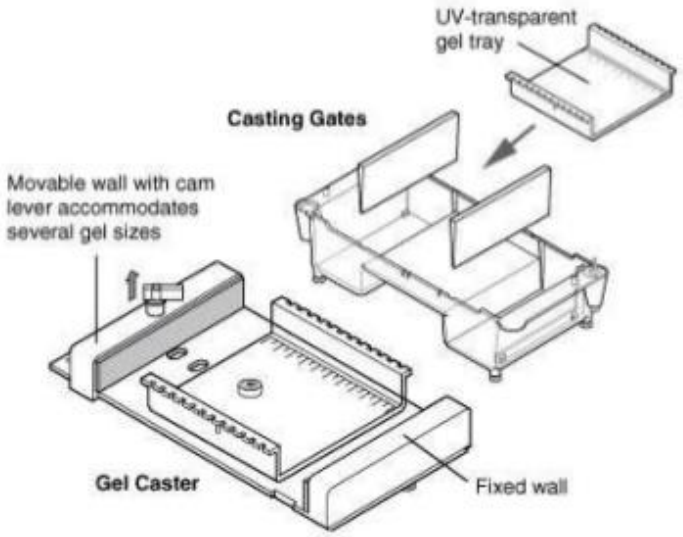
General procedure

Casting of gel

The gel is prepared by dissolving the agarose powder in an appropriate buffer, such as TAE or TBE, to be used in electrophoresis. The agarose is dispersed in the buffer before heating it to near-boiling point, but avoid boiling. The melted agarose is allowed to cool sufficiently before pouring the solution into a cast as the cast may warp or crack if the agarose solution is too hot. A comb is placed in the cast to create wells for loading sample, and the gel should be completely set before use.

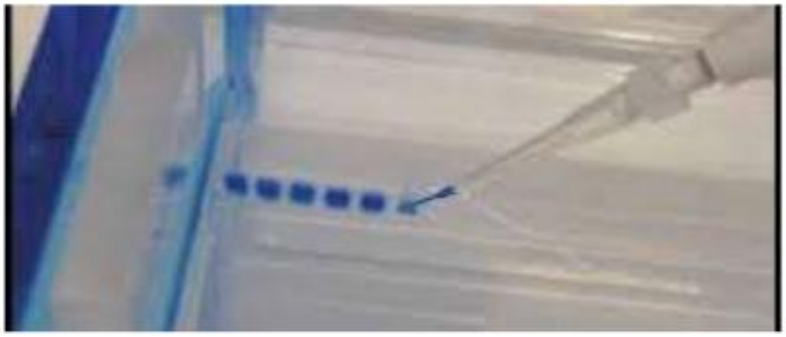
The concentration of gel affects the resolution of DNA separation. For a standard agarose gel electrophoresis, a 0.8% gives good separation or resolution of large 5-10kb DNA fragments, while 2% gel gives good resolution for small 0.2-1kb fragments. 1% gels are common for many applications


7. 18. 2004


UV-transparent
gel tray
Casting Gates
Movable wall with cam
lever accommodates
several gel sizes
Gel Caster
Fixed wall

Loading of samples

Once the gel has set, the comb is removed, leaving wells where DNA samples can be loaded. Loading buffer is mixed with the DNA sample before the mixture is loaded into the wells. The loading buffer contains a dense compound, which may be glycerol, sucrose, or Ficoll, that raises the density of the sample so that the DNA sample may sink to the bottom of the well. If the DNA sample contains residual ethanol after its preparation, it may float out of the well. The loading buffer also include colored dyes such as xylene cyanol and bromophenol blue used to monitor the progress of the electrophoresis. The DNA samples are loaded using a micropipette.



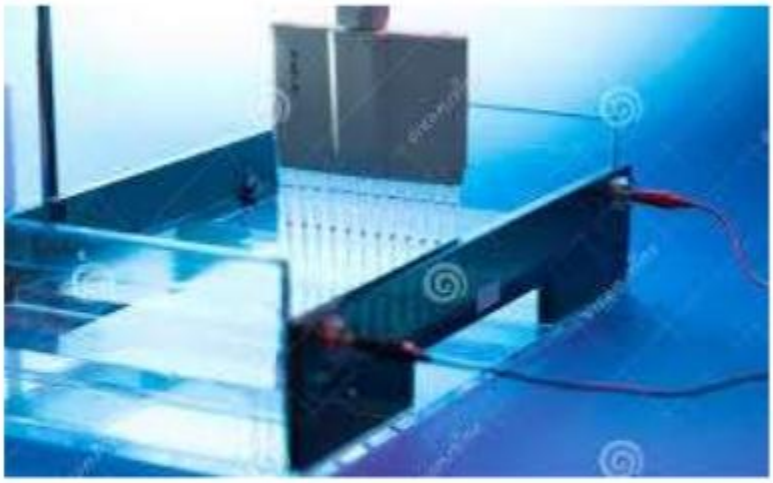
Electrophoresis

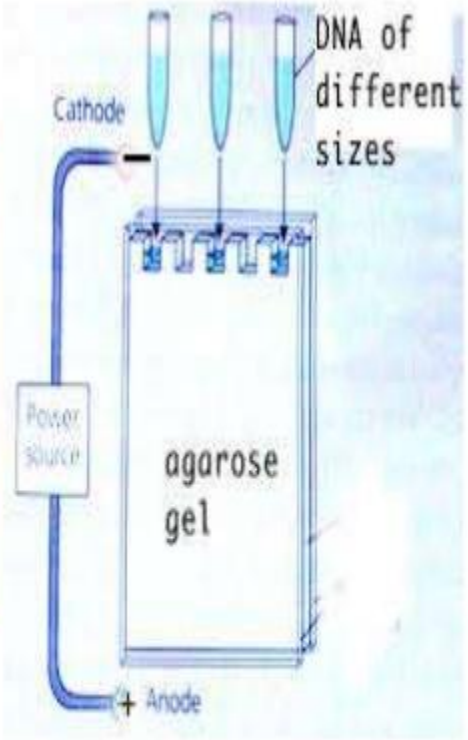
Agarose gel electrophoresis is most commonly done horizontally in a submarine mode whereby the slab gel is completely submerged in buffer during electrophoresis.

For optimal resolution of DNA greater than 2 kb in size in standard gel electrophoresis, 5 to 8 V/cm is recommended (the distance in cm refers to the distance between electrodes, therefore this recommended voltage would be 5 to 8 multiplied by the distance between the electrodes in cm). Voltage may also be limited by the fact that it heats the gel and may cause the gel to melt if it is run at high voltage for a prolonged period, especially if the gel used is LMP agarose gel.

Too high a voltage may also reduce resolution, as well as causing band streaking for large DNA molecules. Too low a voltage may lead to broadening of band for small DNA fragments due to dispersion and diffusion.

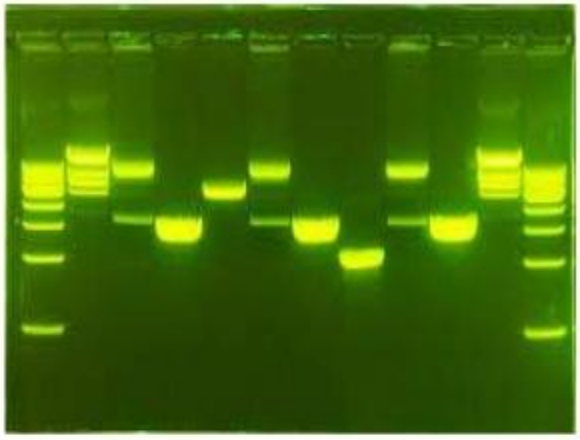
A DNA marker is also run together for the estimation of the molecular weight of the DNA fragments.

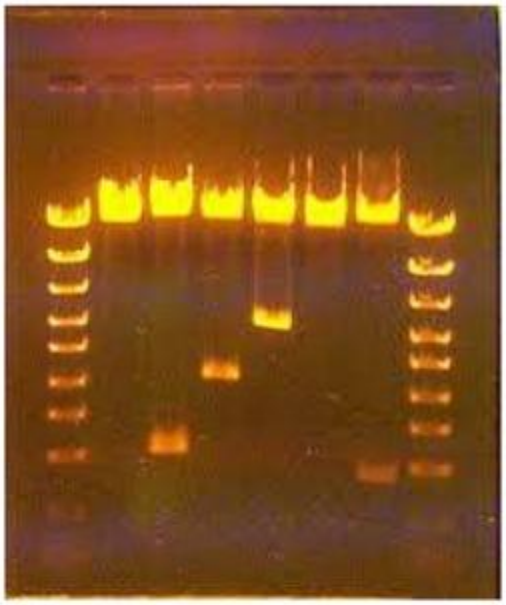




Visualization / Staining

DNA as well as RNA is normally visualized by staining with ethidium bromide, which intercalates into the major grooves of the DNA and fluoresces under UV light. The ethidium bromide may be added to the agarose solution before it gels, or the DNA gel may be stained later after electrophoresis. Destaining of the gel is not necessary but may produce better images.





Other methods of staining are available;

examples are SYBR Green, GelRed, methylene blue, brilliant cresyl blue, Nile bluesulphate, and crystal violet. SYBR Green, GelRed and other similar commercial products are sold as safer alternatives to ethidium bromide as it has been shown to be mutagenic in Ames test, although the carcinogenicity of ethidium bromide has not actually been established. SYBR Green requires the use of a blue-light transilluminator. DNA stained with crystal violet can be viewed under natural light without the use of a UV transilluminator which is an advantage; however it may not produce a strong band.

When stained with ethidium bromide, the gel is viewed with an ultraviolet (UV) transilluminator.

Standard transilluminators use wavelengths of 302/312-nm UV-B. The transilluminator apparatus may also contain image capture devices, such as a digital or Polaroid camera that allow an image of the gel to be taken or printed.

Applications

- Estimation of the size of DNA molecules following restriction enzyme digestion, e.g. in restriction mapping of cloned DNA.
- Analysis of PCR products, e.g. in molecular genetic diagnosis or genetic fingerprinting
- Separation of restricted genomic DNA prior to Southern transfer or of RNA prior to Northern transfer.

❒ Agarose gel electrophoresis is a method of gel electrophoresis used in biochemistry, molecular biology, and clinical chemistry to separate a mixed population of DNA or proteins in a matrix of agarose.

❒ The proteins may be separated by charge and/or size (IEF agarose, essentially size independent), and the DNA and RNA fragments by length.

❒ Biomolecules are separated by applying an electric field to move the charged molecules through an agarose matrix, and the biomolecules are separated by size in the agarose gel matrix.