**Lec.15: Bioseparation Technique**

**Gel electrophoresis(Composition)**

***The matrix:***

**The matrix used to contain, and then separate the target molecules. In most cases, the gel is a [crosslinked polymer](https://en.wikipedia.org/wiki/Crosslinked_polymer%22%20%5Co%20%22Crosslinked%20polymer) whose composition and porosity is chosen based on the specific weight and composition of the target molecules to be analyzed.**

**When separating**[**proteins**](https://en.wikipedia.org/wiki/Protein)**,**[**DNA**](https://en.wikipedia.org/wiki/DNA)**,**[**RNA**](https://en.wikipedia.org/wiki/RNA)**, or [oligonucleotides](https://en.wikipedia.org/wiki/Oligonucleotide%22%20%5Co%20%22Oligonucleotide), the gel is usually composed of different concentrations of [acrylamide](https://en.wikipedia.org/wiki/Acrylamide%22%20%5Co%20%22Acrylamide) and a**[**cross-linker**](https://en.wikipedia.org/wiki/Cross-linker)**, producing different sized mesh networks of polyacrylamide. When separating larger nucleic acids, the preferred matrix is purified agarose. In both cases, the gel forms a solid, yet porous matrix. Acrylamide, in contrast to polyacrylamide, is a**[**neurotoxin**](https://en.wikipedia.org/wiki/Neurotoxin)**and must be handled using appropriate safety precautions to avoid poisoning. Agarose is composed of long unbranched chains of uncharged carbohydrate without cross links resulting in a gel with large pores allowing for the separation of macromolecules and**[**macromolecular complexes**](https://en.wikipedia.org/wiki/Affinity_electrophoresis)**.**

***Types of gel*:**

**The types of gel most typically used are agarose and polyacrylamide gels;each type of gel is well-suited to different types and sizes of analyte.**

***Agarose gels:***

**Agarose gels which are made from the: natural**[**polysaccharide**](https://en.wikipedia.org/wiki/Polysaccharide)[**polymers**](https://en.wikipedia.org/wiki/Polymer)**extracted from**[**seaweed**](https://en.wikipedia.org/wiki/Seaweed) **have lower resolving power for DNA but have greater range of separation. Agarose gel electrophoresis can be used for the separation of DNA fragments ranging from 50**[**base pair**](https://en.wikipedia.org/wiki/Base_pair)**to several megabases .**

**Agarose gels do not have a uniform pore size, but are optimal for electrophoresis of proteins that are larger than 200 kDa. . Most modern DNA separation methods now use agarose gels, except for particularly small DNA fragments. Gel electrophoresis of large**[**DNA**](https://en.wikipedia.org/wiki/DNA)**or**[**RNA**](https://en.wikipedia.org/wiki/RNA)**is usually done by agarose gel electrophoresis.Agarose gels are typically run horizontally in a submarine mode.**

***Polyacrylamide gels:***

**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, also Polyacrylamide gel have very high resolving power for small fragments of DNA (5-500 base pair). Polyacrylamide gels are run in a vertical configuration.**

***Gel conditions:***

**Proteins are denatured using**[**sodium dodecyl sulfate**](https://en.wikipedia.org/wiki/Sodium_dodecyl_sulfate)**, usually as part of the**[**SDS-PAGE**](https://en.wikipedia.org/wiki/Polyacrylamide_gel_electrophoresis)**process. For full denaturation of proteins, it is also necessary to reduce the covalent**[**disulfide bonds**](https://en.wikipedia.org/wiki/Disulfide_bond)**that stabilize their**[**tertiary**](https://en.wikipedia.org/wiki/Tertiary_structure)**and**[**quaternary structure**](https://en.wikipedia.org/wiki/Quaternary_structure)**, reducing conditions are usually maintained by the addition of**[**β-mercaptoethanol**](https://en.wikipedia.org/wiki/Beta-mercaptoethanol)**.**

***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.**

**After the electrophoresis is complete, the molecules in the gel can be**[**stained**](https://en.wikipedia.org/wiki/Staining_%28biology%29)**to make them visible. DNA may be visualized using [ethidium bromide](https://en.wikipedia.org/wiki/Ethidium_bromide%22%20%5Co%20%22Ethidium%20bromide) ,**[**fluoresce**](https://en.wikipedia.org/wiki/Fluorescence)**under**[**ultraviolet**](https://en.wikipedia.org/wiki/Ultraviolet)**light, while protein may be visualised using** [**Coomassie Brilliant Blue**](https://en.wikipedia.org/wiki/Coomassie_Brilliant_Blue)**dye.**

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**After separation, the gel will then be physically cut, and the protein complexes extracted from each portion separately,each extract may then be analyzed, this can provide a great deal of information about the proteins in a complex.**

***Applications:***

**Estimation of the size of DNA molecules following restriction enzyme #**

* **Analysis of**[**PCR**](https://en.wikipedia.org/wiki/PCR)**products**
* **# Separation of restricted genomic DNA.**

**# Gel electrophoresis is used in**[**forensics**](https://en.wikipedia.org/wiki/Forensic_chemistry)**,**[**molecular biology**](https://en.wikipedia.org/wiki/Molecular_biology)**,**[**genetics**](https://en.wikipedia.org/wiki/Genetics)**,**[**microbiology**](https://en.wikipedia.org/wiki/Microbiology)**and**[**biochemistry**](https://en.wikipedia.org/wiki/Biochemistry)**.**