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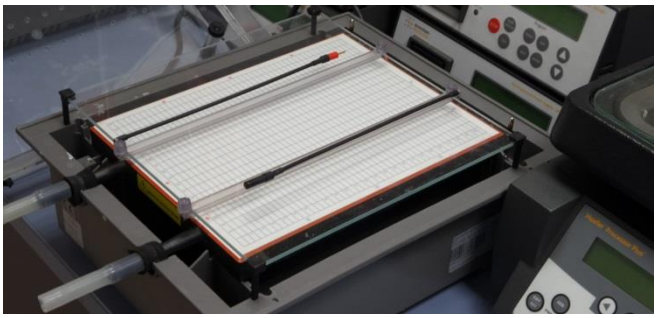
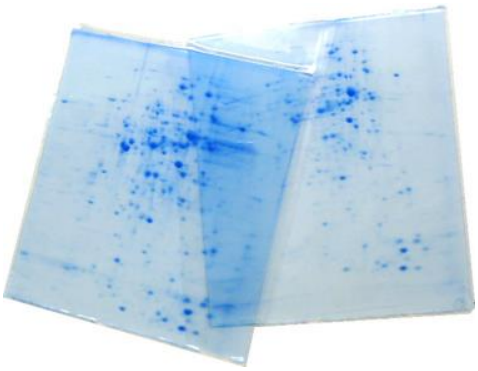
Electrophoresis Systems Laboratory (ESL)

Electrophoresis is the general name of the techniques based on seperation of macromolecules like protein, DNA and RNA under an electrical field effect. These macromolecules runs in jel matrices at varying velocities dependent on the charge they carry. The size and the shape of the macromolecule exerts a resistive force during the passage of the molecule through the matrix pores, hence the size of the molecule determines its velocity along with its shape and charge. Consequently each type of molecule moves at different velocities and they seperate from each other. Finally the gel is immersed in staining solution to label those macromolecules. Depending on molecule of interest comassie blue, silver nitrate or SYBR greenmight be utilized. In our laboratory we have vertical electrophoresis systems (Amersham, Biorad), a Pulsed Field Gel Elecrophoresis System (Amersham), Isoelectric Focusing System (Biorad) and a 2 Dimensional Gel Electrohphoresis System (Amersham, Biorad).

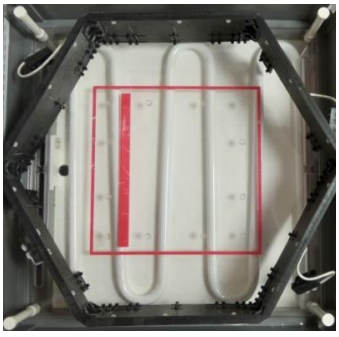


Vertical Electrophoresis System (VGE): It is used for separation of peptide and proteins on the basis of their molecular weight. Proteins are negatively charged following a denaturation with SDS and they are forced to move in a gel matrix where they all have the same molecular weight/charge ratio. They only differ in resistive force applied to them that is linerarly dependent on their molecular size. There is also Native-PAGE analysises where the 3 dimensional structure of the protein is conserved and the charge of the protein is also a parameter affecting the movement of the molecule. In any case molecular weight can be determined from samples concentrated as low as nanograms/liter to miligrams/liter.



Isoelectric Focusing System(IEF): The Isoelectric Point (pI) the macromolecules are determined by this system. It is based on a technique where proteins moved on a pH gradient gel under an electric field. Each type of molecule moves to a pH region where its total net charge becomes zero. This point is called Isoelectric Point and denoted by pI. Depending on protein concentration the gel might be stained by comassie blue or silver nitrate.

Pulsed Field Gel Electrophoresis System(PFGE): Seperation of DNA molecules with molecular weight greater than 20 kb is accomplished by this technique. Different from a regular agarose gel electrophoresis system, the direction of the electrical field is altered between 3 different directions at varying frequencies, consequently a better separation is achieved between DNA molecules with molecular weight close to each other or greater than 20 kb up to 8 Mb. This technique is occasionally used for classification the species by their DNA fragments.



2 Dimensional Gel Electrophoresis System (TDGE): IEF and Vertical Electrophoresis systems are used sequentially to create a 2 dimensional gel image where Isoelectric Point (pI) and Molecular Weight of the proteins might be observed on a single gel. Samples in the range of nanograms/liter to miligrams/liter might be characterized by different staining techniques.

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