SOUTHERN BLOTTING

Q.What is electrophoresis?

The term electrophoresis describes the migration of a charged particle under the influence of an electric field. *Many biological molecules such as amino acids, peptides, proteins, nucleotides and nucleic acids possess ionisable groups and therefore at any pH, exist in solution as electrically charged species either as cations (+) or anions (-).* Under the influence of an electric field these charged particles will migrate either to the cathode or to the anode, depending on the nature of their net charge.

Q.What do you mean by vertical gel?

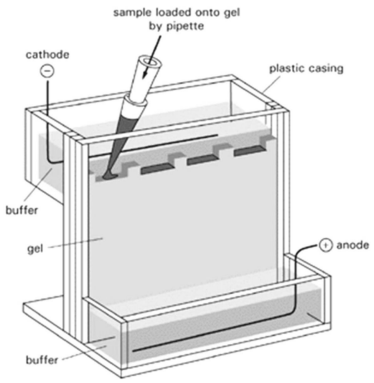
i)Vertical slab gel units used to separate **protein** in acrylamide gel.

ii)The gel is formed between the two glass plates that are clumped together but held apart by plastic spacers.

iii)Gel dimensions are typically 12 cm × 14cm, with a thickness of 1-2mm.

iv)A plastic comb is placed in the gel solution ans is removed after polymerization to provide loading wells for samples.

v)When the apparatus is assembled, the lower electrophoresis tank buffer surrounds the gel plates and affords sme cooling of the gel plate.



Q.What do you mean by horizontal gel system?

i)The gel cast on a glass or plastic sheet and placed on a cooling plate. (an insulated surface through which cooling water is passed to conduct away generated heat).

ii)Connection between the gel and electrode buffer is made using a thick wad of wetted filter paper (*however, agarose gels for DNA electrophoresis are sun submerged in the buffer*)

iii)The powerpack supplies a direct cxurrent between the electrodes in the electrophoresis.

iv)All electrophoresis is carried out in an appropriate buffer, which is essential to maintain a constant state of ionization of the molecules being separated.

Any variation in pH would alter the overall charge and hence the mobilities .

Q.What is the purpose of using southern blotting technique? Who invented this technique?

A mixture of DNA, RNA or protein fragments can be separated by gel electrophoresis and the separated bands can be stained and visualized directly in the gel. Frequently it is necessary to know what sequences in a DNA restriction fragment are transcribed into RNA or to be able to map sequences by hybridization to restriction fragments. This can be done by a neat method, Southern blotting.

Edward Southern (1975, 1979b) evolved this procedure.