BLOTTING TECHNIQUES

Blotting refers to the technique, where molecules that have been separated by electrophoresis are transferred or blotted onto a specific type of paper usually nitrocellulose or PVDF by the application of an electrical current (electroblotting). There are three types of blotting techniques in current use - Southern, northern and western and are used to detect DNA, messenger RNA (mRNA) and protein, respectively.

WESTERN BLOTTING

Once protein samples have been separated, based on molecular mass, charge, pI or a combination of these factors, they are then transferred, usually by electroblotting, from the gel to a nitrocellulose or PVDF membrane. As a result of this process, the proteins are bound to the membrane surface which allows detection by an antibody specific to the protein of interest. However, since the membranes used have a high protein binding capacity, it is essential to block all non-specific protein. This is normally carried out by incubation of the membrane with BSA , non-fat milk powder or casein. Once non-specific sites have been blocked, an antibody specific to the protein of interest can be added. This antibody can be tagged with a label that will allow subsequent detection e.g. horseradish peroxidose or fluorescein. If the primary antibody does not contain a label, a secondary labelled antibody is employed.