**IMMUNOLOGICAL SCREENING**

This technique is used to identify or locate the gene product or protein and it is alternative strategy to Hybridization probing used in southern blotting and northern blotting. In Hybridisation, gene is probed where as in Immunological assay proteins are probed. Immunological techniques therefore presuppose that the cloned gene is being expressed, so that the protein is being made, and that this protein is not normally present in the host cells.

Here antibodies are required which are developed by injecting the pure proteins into the bloodstream rabbit. The rabbit immune system senses it as foreign body or antigen and develops antibodies against the foreign protein.

Purified antibodies can be obtained from a small volume of blood taken from a rabbit injected with the foreign protein.

Now this purified antibody is used to detect protein in recombinant colonies.

There are several ways to perform immunological assay. Most simplest is Colony Hybridisation

Colony Hybridisation- Recombinant Colonies are grown on a petriplates. They are transferred to Nitrocellulose membrane, Cell lysis is carried out and solution containing antibodies specific to recombinant protein are added. These antibodies are primary antibodies. Another set of antibody specific to primary antibodies are added (secondary antibodies), which detects the primary antibody and recombinant protein. The primary antibody—is detected by washing the membrane with a labeled secondary antibody, which binds specifically to the primary antibody. Several secondary antibody molecules can bind to a single primary antibody molecule, increasing the amount of signal that is produced and enabling a clearer detection of each positive colony.

The label can be radioactive which is detected by autoradiography or it can be enzyme linked, non radioactive that gives fluorescent signal.





Following the interaction of the primary antibody with the target protein (antigen), any unbound antibody is washed away, and the matrix is treated with a second antibody (secondary antibody) that is specific for the primary antibody. In many assay systems, the secondary antibody has an enzyme, such as alkaline phosphatase, attached to it. After the matrix is washed, a colorless substrate is added. If the secondary antibody has bound to the primary antibody, the colorless substrate is hydrolyzed by the attached enzyme and produces a colored compound that accumulates at the site of the reaction.

