Blotting techniques

Blotting is the technique in which nucleic acids or proteins are immobilized onto a solid support generally nylon or nitrocellulose membranes. Blotting of nucleic acid is the central technique for hybridization studies. Nucleic acid labeling and hybridization on membranes have formed the basis for a range of experimental techniques involving understanding of gene expression, organization, etc.

Identifying and measuring specific proteins in complex biological mixtures, such as blood, have long been important goals in scientific and diagnostic practice. More recently the identification of abnormal genes in genomic DNA has become increasingly important in clinical research and genetic counseling. Blotting techniques are used to identify unique proteins and nucleic acid sequences. They have been developed to be highly specific and sensitive and have become important tools in both molecular biology and clinical research.

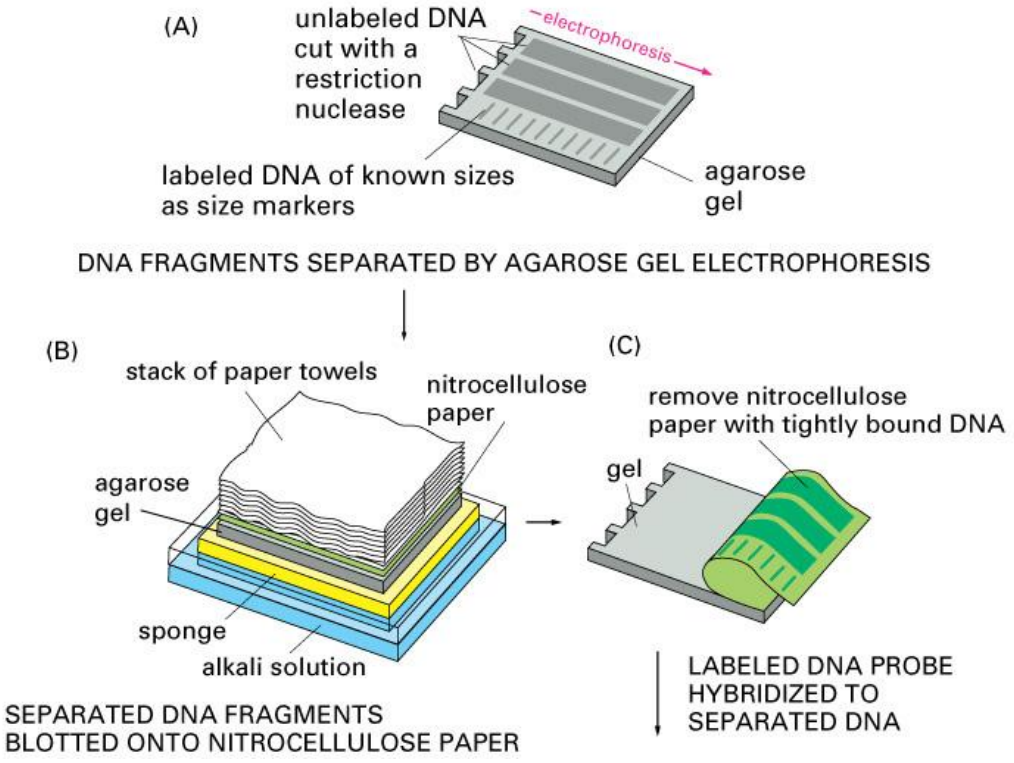
General principle

The blotting methods are fairly simple and usually consist of four separate steps: electrophoretic separation of protein or of nucleic acid fragments in the sample; transfer to and immobilization on paper support; binding of analytical probe to target molecule on paper; and visualization of bound probe. Molecules in a sample are first separated by electrophoresis and then transferred on to an easily handled support medium or membrane. This immobilizes the protein or DNA fragments, provides a faithful replica of the original separation, and facilitates subsequent biochemical analysis. After being transferred to the support medium the immobilized protein or nucleic acid fragment is localized by the use of probes, such as antibodies or DNA, that specifically bind to the molecule of interest. Finally, the position of the probe that is bound to the immobilized target molecule is visualized usually by autoradiography. Three main blotting techniques have been developed and are commonly called Southern, northern and western blotting.

Southern blot

Southern blot is a method used to check for the presence of a DNA sequence in a DNA sample. The method is named after its inventor, the British biologist Edwin Southern.
The procedure for Southern blot technique is as detailed below:

- Restriction endonucleases are used to cut high-molecular-weight DNA strands into smaller fragments, which are then electrophoresed on an agarose gel to separate them by size.
- If the DNA fragments are larger than 15 kb, then prior to blotting, the gel may be treated with an acid, such as dilute HCl, which depurinates the DNA fragments, breaking the DNA into smaller pieces, thus allowing more efficient transfer from the gel to membrane.
- If alkaline transfer methods are used, the DNA gel is placed into an alkaline solution (containing NaOH) to denature the double-stranded DNA. The denaturation in an alkaline environment may improve binding of the negatively charged DNA to a positively charged membrane, separating it into single DNA strands for later hybridization to the probe and destroys any residual RNA that may still be present in the DNA.
- A sheet of nitrocellulose (or nylon) membrane is placed on top of (or below, depending on the direction of the transfer) the gel. Pressure is applied evenly to the gel (either using suction, or by placing a stack of paper towels and a weight on top of the membrane and gel), to ensure good and even contact between gel and membrane. Buffer transfer by capillary action from a region of high water potential to a region of low water potential (usually filter paper and paper tissues) is used to move the DNA from the gel on to the membrane; ion exchange interactions bind the DNA to the membrane due to the negative charge of the DNA and positive charge of the membrane.
- The membrane is then baked in a vacuum or regular oven at 80 °C for 2 hours or exposed to ultraviolet radiation (nylon membrane) to permanently attach the transferred DNA to the membrane.
- The membrane is then exposed to a hybridization probe—a single DNA fragment with a specific sequence whose presence in the target DNA is to be determined. The probe DNA is labelled so that it can be detected, usually by incorporating radioactivity or tagging the molecule with a fluorescent or chromogenic dye.
- After hybridization, excess probe is washed from the membrane and the pattern of hybridization is visualized on X-ray film by autoradiography in the case of a radioactive or fluorescent probe, or by development of colour on the membrane if a chromogenic detection method is used.



Hybridization of the probe to a specific DNA fragment on the filter membrane indicates that this fragment contains DNA sequence that is complementary to the probe. The transfer step of the DNA from the electrophoresis gel to a membrane permits easy binding of the labeled hybridization probe to the size-fractionated DNA. Southern blots performed with restriction enzyme-digested genomic DNA may be used to determine the number of sequences (e.g., gene copies) in a genome. A probe that hybridizes only to a single DNA segment that has not been cut by the restriction enzyme will produce a single band on a Southern blot, whereas multiple bands will likely be observed when the probe hybridizes to several highly similar sequences (e.g., those that may be the result of sequence duplication). Modification of the hybridization conditions (ie, increasing the hybridization temperature or decreasing salt concentration) may be used to increase specificity and decrease hybridization of the probe to sequences that are less than 100% similar.

Northern blot

The **northern blot** technique is used to study gene expression by detection of RNA (or isolated mRNA) in a sample. With northern blotting it is possible to observe cellular control over structure and function by determining the particular gene expression levels during differentiation,

morphogenesis, as well as abnormal or diseased conditions. This technique was developed in 1977 by James Alwine, David Kemp and George Stark at Stanford University. Northern blotting takes its name from its similarity to the first blotting technique, the Southern blot. The major difference is that RNA, rather than DNA, is analyzed in the northern blot.

Procedure

The blotting procedure starts with extraction of total RNA from a homogenized tissue sample. The mRNA can then be isolated through the use of oligo (dT) cellulose chromatography to maintain only those RNAs with a poly(A) tail. RNA samples are then separated by gel electrophoresis. A nylon membrane with a positive charge is the most effective for use in northern blotting since the negatively charged nucleic acids have a high affinity for them. The transfer buffer used for the blotting usually contains formamide because it lowers the annealing temperature of the probe-RNA interaction preventing RNA degradation by high temperatures. Once the RNA has been transferred to the membrane it is immobilized through covalent linkage to the membrane by UV light or heat. After a probe has been labeled, it is hybridized to the RNA on the membrane. The membrane is washed to ensure that the probe has bound specifically. The hybrid signals are then detected by X-ray film and can be quantified by densitometry.

Applications

Northern blotting allows in observing a particular gene's expression pattern between tissues, organs, developmental stages, environmental stress levels, pathogen infection. The technique has been used to show over expression of oncogenes and down regulation of tumor-suppressor genes in cancerous cells when compared to 'normal' tissue, as well as the gene expression in the rejection of transplanted organs. If an up regulated gene is observed by an abundance of mRNA on the northern blot the sample can then be sequenced to determine if the gene is known to researchers or if it is a novel finding. The expression patterns obtained under given conditions can provide insight into the function of that gene. Since the RNA is first separated by size, if only one probe type is used variance in the level of each band on the membrane can provide insight into the size of the product, suggesting alternative splice products of the same gene or repetitive sequence motifs. The variance in size of a gene product can also indicate deletions or errors in transcript processing, by altering the probe target used along the known sequence it is possible to determine which region of the RNA is missing.

Advantages & disadvantages

Analysis of gene expression can be done by several different methods including RT-PCR, RNase protection assays, microarrays, serial analysis of gene expression (SAGE), as well as northern blotting. Microarrays are quite commonly used and are usually consistent with data obtained from northern blots, however at times northern blotting is able to detect small changes in gene expression that microarrays cannot. The advantage that microarrays have over northern blots is that thousands of genes can be visualized at a time while northern blotting is usually looking at one or a small number of genes. A problem in northern blotting is often sample degradation by RNases (both endogenous to the sample and through environmental contamination) which can be avoided by proper sterilization of glassware and the use of RNase inhibitors such as DEPC (diethylpyrocarbonate). The chemicals used in most northern blots can be a risk to the researcher, since formaldehyde, radioactive material; ethidium bromide, DEPC, and UV light are all harmful under certain exposures. Compared to RT-PCR northern blotting has a low sensitivity but it also has a high specificity which is important to reduce false positive results. The advantages of using northern blotting include the detection of RNA size, the observation of alternate splice products, the use of probes with partial homology, the quality and quantity of RNA can be measured on the gel prior to blotting, and the membranes can be stored and reprobed for years after blotting.

Reverse northern blot

A variant of the procedure known as the reverse northern blot is occasionally used. In this procedure, the substrate nucleic acid (that is affixed to the membrane) is a collection of isolated DNA fragments, and the probe is RNA extracted from a tissue and radioactively labelled. The use of DNA microarrays that have come into widespread use in the late 1990s and early 2000s is more akin to the reverse procedure, in that they involve the use of isolated DNA fragments affixed to a substrate, and hybridization with a probe made from cellular RNA. Thus the reverse procedure, though originally uncommon, enabled northern analysis to evolve into gene expression profiling, in which many (possibly all) of the genes in an organism may have their expression monitored.

Western blot

The **western blot** (alternatively, **immunoblot**) is used to detect specific proteins in a given sample of tissue homogenate or extract. The method originated from the laboratory of George Stark at Stanford. The name **western blot** was given to the technique by W. Neal Burnette.

Steps in a western blot

Tissue preparation

Samples may be taken from whole tissue or from cell culture. In most cases, solid tissues are first broken down mechanically using a blender (for larger sample volumes), homogenizer (smaller volumes) or sonication. Assorted detergents, salts and buffers may be employed to encourage lysis of cells and to solubilize proteins. Protease and phosphatase inhibitors are often added to prevent the digestion of the sample by its own enzymes. Tissue preparation is often done at cold temperatures to avoid protein denaturing. A combination of biochemical and mechanical techniques, including various types of filtration and centrifugation can be used to separate different cell compartments and organelles.

Gel electrophoresis

The proteins of the sample are separated using gel electrophoresis. Separation of proteins may be by isoelectric point (pI), molecular weight, electric charge or a combination of these factors. SDS-PAGE (SDS polyacrylamide gel electrophoresis) maintains polypeptides in a denatured state once they have been treated with strong reducing agents to remove secondary and tertiary structure and thus allows separation of proteins by their molecular weight. Sampled proteins become covered in the negatively charged SDS and move to the positively charged electrode

through the acrylamide mesh of the gel. Smaller proteins migrate faster through this mesh and the proteins are thus separated according to size. The concentration of acrylamide determines the resolution of the gel - the greater the acrylamide concentration the better the resolution of lower molecular weight proteins. The lower the acrylamide concentration the better the resolution of higher molecular weight proteins. Proteins travel only in one dimension along the gel for most blots.

Samples are loaded into wells in the gel. One lane is usually reserved for a *marker* or *ladder*, a commercially available mixture of proteins having defined molecular weights, typically stained so as to form visible, coloured bands. When voltage is applied along the gel, proteins migrate into it at different speeds. These different rates of advancement separate into *bands* within each lane. It is also possible to use a two-dimensional (2-D) gel which spreads the proteins from a single sample out in two dimensions. Proteins are separated according to isoelectric point (pH at which they have neutral net charge) in the first dimension, and according to their molecular weight in the second dimension.

Transfer

In order to make the proteins accessible to antibody detection, they are moved from within the gel onto a nitrocellulose or polyvinylidene difluoride (PVDF) membrane similar to Southern blot DNA transfer. Another method for transferring the proteins is called electro blotting and uses an electric current to pull proteins from the gel into the PVDF or nitrocellulose membrane. The proteins move from within the gel onto the membrane while maintaining the organization they had within the gel. As a result of this "blotting" process, the proteins are exposed on a thin surface layer for detection. Protein binding is based upon hydrophobic interactions, as well as charged interactions between the membrane and protein. Nitrocellulose membranes are cheaper than PVDF, but are far more fragile and do not stand up well to repeated probings.

The uniformity and overall effectiveness of transfer of protein from the gel to the membrane can be checked by staining the membrane with Coomassie or Ponceau S dyes. Ponceau S is the more common of the two, due to Ponceau S's higher sensitivity and its water solubility makes it easier to subsequently destain and probe the membrane.

Blocking

Since the membrane has been chosen for its ability to bind protein and both antibodies and the target are proteins, steps must be taken to prevent interactions between the membrane and the

antibody used for detection of the target protein. Blocking of non-specific binding is achieved by placing the membrane in a dilute solution of protein - typically Bovine serum albumin (BSA) or non-fat dry milk (both are inexpensive), with a minute percentage of detergent such as Tween 20. The protein in the dilute solution attaches to the membrane in all places where the target proteins have not attached. Thus, when the antibody is added, there is no room on the membrane for it to attach other than on the binding sites of the specific target protein. This reduces "noise" in the final product of the Western blot, leading to clearer results, and eliminates false positives.

Detection

During the detection process the membrane is "probed" for the protein of interest with a modified antibody which is linked to a reporter enzyme, which when exposed to an appropriate substrate drives a colourimetric reaction and produces a colour. For a variety of reasons, this traditionally takes place in a two-step process, although there are now one-step detection methods available for certain applications.

Two step

- Primary antibody

Antibodies are generated when a host species or immune cell culture is exposed to the protein of interest. Normally, this is part of the immune response; whereas here they are harvested and used as sensitive and specific detection tools that bind the protein directly.

After blocking, a dilute solution of primary antibody (generally between 0.5 and 5 micrograms/ml) is incubated with the membrane under gentle agitation. Typically, the solution is composed of buffered saline solution with a small percentage of detergent, and sometimes with powdered milk or BSA. The antibody solution and the membrane can be sealed and incubated together for anywhere from 30 minutes to overnight. It can also be incubated at different temperatures, with warmer temperatures being associated with more binding, both specific (to the target protein, the "signal") and non-specific ("noise").

- Secondary antibody

After rinsing the membrane to remove unbound primary antibody, the membrane is exposed to another antibody, directed at a species-specific portion of the primary antibody. This is known as a secondary antibody, and due to its targeting properties, tends to be referred to as "anti-mouse," "anti-goat," etc. Antibodies come from animal sources (or animal sourced hybridoma cultures); an anti-mouse secondary will bind to just about any mouse-sourced primary antibody.

This allows some cost savings by allowing an entire lab to share a single source of mass-produced antibody, and provides far more consistent results. The secondary antibody is usually linked to biotin or to a reporter enzyme such as alkaline phosphatase or horseradish peroxidase. This means that several secondary antibodies will bind to one primary antibody and enhance the signal.

Most commonly, a horseradish peroxidase-linked secondary is used in conjunction with a chemiluminescent agent, and the reaction product produces luminescence in proportion to the amount of protein. A sensitive sheet of photographic film is placed against the membrane, and exposure to the light from the reaction creates an image of the antibodies bound to the blot. A cheaper but less sensitive approach utilizes a 4-chloronaphthol stain with 1% hydrogen peroxide; reaction of peroxide radicals with 4-chloronaphthol produces a dark brown stain that can be photographed without using specialized photographic film.

As with the ELISPOT and ELISA procedures, the enzyme can be provided with a substrate molecule that will be converted by the enzyme to a colored reaction product that will be visible on the membrane (see the figure below with blue bands).
A third alternative is to use a radioactive label rather than an enzyme coupled to the secondary antibody, such as labeling an antibody-binding protein like *Staphylococcus* Protein A with a radioactive isotope of iodine. Since other methods are safer, quicker and cheaper this method is now rarely used.

One step

Historically, the probing process was performed in two steps because of the relative ease of producing primary and secondary antibodies in separate processes. This gives researchers and corporations huge advantages in terms of flexibility, and adds an amplification step to the detection process. Given the advent of high-throughput protein analysis and lower limits of detection, however, there has been interest in developing one-step probing systems that would allow the process to occur faster and with less consumables. This requires a probe antibody which both recognizes the protein of interest and contains a detectable label, probes which are often available for known protein tags. The primary probe is incubated with the membrane in a manner similar to that for the primary antibody in a two-step process, and then is ready for direct detection after a series of wash steps.

Analysis

After the unbound probes are washed away, the western blot is ready for detection of the probes that are labeled and bound to the protein of interest. In practical terms, not all westerns reveal protein only at one band in a membrane. Size approximations are taken by comparing the stained bands to that of the marker or ladder loaded during electrophoresis. The process is repeated for a structural protein, such as actin or tubulin, that should not change between samples. The amount of target protein is indexed to the structural protein to control between groups. This practice ensures correction for the amount of total protein on the membrane in case of errors or incomplete transfers.

Colorimetric detection

The colorimetric detection method depends on incubation of the western blot with a substrate that reacts with the reporter enzyme (such as peroxidase) that is bound to the secondary antibody. This converts the soluble dye into an insoluble form of a different color that precipitates next to the enzyme and thereby stains the membrane. Development of the blot is then stopped by washing away the soluble dye. Protein levels are evaluated through densitometry (how intense the stain is) or spectrophotometry.

Chemiluminescent detection

Chemiluminescent detection methods depend on incubation of the western blot with a substrate that will luminesce when exposed to the reporter on the secondary antibody. The light is then detected by photographic film, and more recently by CCD cameras which captures a digital image of the western blot. The image is analysed by densitometry, which evaluates the relative amount of protein staining and quantifies the results in terms of optical density. Newer software allows further data analysis such as molecular weight analysis if appropriate standards are used.

Radioactive detection

Radioactive labels do not require enzyme substrates, but rather allow the placement of medical X-ray film directly against the western blot which develops as it is exposed to the label and creates dark regions which correspond to the protein bands of interest (see image to the right). The importance of radioactive detections methods is declining, because it is very expensive, health and safety risks are high and ECL provides a useful alternative.

Fluorescent detection

The fluorescently labeled probe is excited by light and the emission of the excitation is then detected by a photosensor such as CCD camera equipped with appropriate emission filters which captures a digital image of the western blot and allows further data analysis such as molecular weight analysis and a quantitative western blot analysis. Fluorescence is considered to be among the most sensitive detection methods for blotting analysis.

Secondary probing

One major difference between nitrocellulose and PVDF membranes relates to the ability of each to support "stripping" antibodies off and reusing the membrane for subsequent antibody probes. While there are well-established protocols available for stripping nitrocellulose membranes, the sturdier PVDF allows for easier stripping, and for more reuse before background noise limits experiments. Another difference is that, unlike nitrocellulose, PVDF must be soaked in 95% ethanol, isopropanol or methanol before use. PVDF membranes also tend to be thicker and more resistant to damage during use.

2-D Gel Electrophoresis

2-dimensional SDS-PAGE uses the principles and techniques outlined above. 2-D SDS-PAGE, as the name suggests, involves the migration of polypeptides in 2 dimensions. For example, in the first dimension polypeptides are separated according to isoelectric point, while in the second dimension polypeptides are separated according to their molecular weight. The isoelectric point of a given protein is determined by the relative number of positively (e.g. lysine and arginine) and negatively (e.g. glutamate and aspartate) charged amino acids, with negatively charged amino acids contributing to a high isoelectric point and positively charged amino acids contributing to a low isoelectric point. Samples could also be separated first under nonreducing conditions using SDS-PAGE and under reducing conditions in the second dimension, which breaks apart disulfide bonds that hold subunits together. SDS-PAGE might also be coupled with urea-PAGE for a 2-dimensional gel.

In principle, this method allows for the separation of all cellular proteins on a single large gel. A major advantage of this method is that it often distinguishes between different isoforms of a particular protein - e.g. a protein that has been phosphorylated (by addition of a negatively charged group). Proteins that have been separated can be cut out of the gel and then analysed by mass spectrometry, which identifies the protein.

Eastern blotting

It is a technique to detect protein post translational modification and is an extension of the biochemical technique of western blotting. Proteins blotted from two dimensional SDS-PAGE gel on to a PVDF or nitrocellulose membrane are analyzed for post-translational protein modifications using probes specifically designed to detect lipids, carbohydrate, phospho-moieties or any other protein modification.

The technique was developed to detect protein modifications in two species of *Ehrlichia*- *E. muris* and IOE. Cholera toxin B subunit (which detects lipids), Concanavalin A (which detects glucose moieties) and nitrophospho molybdate-methyl green (detects phosphoproteins) were used to detect protein modifications. The technique showed that the antigenic proteins of the non-virulent *E.muris* are more post-translationally modified than the highly virulent IOE.

The technique was conceptualized by S. Thomas while working on sandal spike phytoplasma and developed at the Dept. of Pathology, University of Texas Medical Branch, Galveston, Texas, while working on the intracellular bacteria, *Ehrlichia.*

Significance

Most of the proteins that are translated from mRNA undergo modifications before becoming functional in cells. The modifications collectively, are known as post-translational modifications (PTMs). The nascent or folded proteins, which are stable under physiological conditions, are then subjected to a battery of specific enzyme-catalyzed modifications on the side chains or backbones.

Post-translational protein modifications includes: acetylation, acylation (myristoylation, palmitoylation), alkylation, arginylation, biotinylation, formylation, glutamylation, glycosylation, glycylation, hydroxylation, isoprenylation, lipoylation, methylation, nitroalkylation, phosphopantetheinylation, phosphorylation, prenylation, selenation, S-nitrosylation, sulfation, transglutamination and ubiquitination (sumoylation).

Post-translational modifications occurring at the N-terminus of the amino acid chain play an important role in translocation across biological membranes. These include secretory proteins in prokaryotes and eukaryotes and also proteins that are intended to be incorporated in various

cellular and organelle membranes such as lysosomes, chloroplast, mitochondria and plasma membranes. Expression of post translated proteins is important in several diseases.

Applications of Blotting and Hybridization Techniques

1. Southern blotting technique is widely used to find specific nucleic acid sequence present in different plant species.
2. Northern blotting technique is widely used to find gene expression and regulation of specific genes.
3. By using blotting technique we can identify infectious agents present in the sample.
4. We can identify inherited disease.
5. It can be applied to mapping restriction sites in single copy gene.

Disadvantages of Blotting and Hybridization Techniques

1. The process is a complex, cumbersome and time consuming one.
2. It requires electrophoretic separation.
3. Only one gene or RNA can be analysed at a time.
4. Gives information about presence of DNA, RNA or proteins but does not give information about regulation and gene interaction.

Dot Blotting Techniques - The drawbacks of blotting techniques have lead to the development of dot blotting technique which is more advanced, less time consuming, accurate and applicable to a wide variety of gene/source simultaneously.

The dot or slot blotting technique is the most widely used of all techniques for analysing. None of the blot methods require electrophoresis prior to blotting and hybridization. Hybridization of cloned DNA without electrophoretic separation is called as dot blotting.

Plaque or Colony Blotting Techniques - This method was first developed by Granstiens and Hogness (1975). This method is used to identify which colony of bacteria contains the DNA of interest among thousands. In this procedure, the bacterial colonies to be screened are transferred onto nitrocellulose or nylon membrane by using replica plating.

Due to the negative charge of the cell surface, some cells bind to the nitrocellulose membrane. Then the membrane is placed in a solution of 0.5 N NaOH to break the cell surface, convert

dsDNA to ssDNA and to bind DNA to the membrane. Later, the membrane is transferred to a solution containing protease solution after neutralizing with neutralization solution.

The DNA is fixed tightly to membrane by either W cross linking or oven baking. This membrane is used for hybridization with a probe and analysed by using autoradiography or biotin method for positive hybridization. A colony whose DNA print (as replica plating provides a replica print master plate colony on the membrane) gives a positive hybridization can be picked from the master plate.

Plaque blotting is similar to colony blotting; the only difference is that instead of bacterial colony, a plaque is transferred onto the membrane. Benton and Davis developed this method in 1977. The greatest advantage of this method is that several identical DNA prints can be easily made from a single master plate containing bacteria/plaques which are to be made.

Dot Plot Assay Techniques - This method is widely used to hybridize DNA from a single cell type against a wide variety of probes, for example, for a viral infection which cannot be identified by normal conventional methods or if we want to know what all genes are expressed in a single cell type (e.g. brain cell).

Cell type or cells that are to be screened are placed on the membrane as 'dot' in the order of rows and columns. Then the cells are denatured by using enzymes or detergents (SDS) and DNA is fixed by using W - cross link or oven baking. This membrane is then used for hybridization by using probes (which are specific to a gene).

Questions

1. The technique in which nucleic acids or proteins are immobilized onto a solid support is called as ..…..

a). Blotting b). Hybridisation
c). Immobilization d). None of the above

2. Blotting techniques are used to identify.....

a). Unique proteins b). Nucleic acid sequences
c). Both a and b d). None of the above

3. Blotting techniques consist of..... separate steps.

a). 4 b). 3
c). 5 d). None of the above

4. Blotting techniques consist of..... separate steps.

a). 4 b). 3
c). 5 d). None of the above

5. Southern blot is a method used to check for the presence of.....

a). DNA b). RNA
c). Protein d). None of the above

6. Southern blot was invented by .....

a). Edwin Southern b). James Alwine
c). David Kemp d). None of the above

7. Northern blot was invented by .....

a). George Stark b). James Alwine
c). David Kemp **d). All the above**

8. Northern blot is a method used to check for the presence of.....

a). DNA **b). RNA**
c). Protein d). None of the above

8. Northern blot is used.....

a). Overexpression of oncogenes
b). Downregulation of tumor-suppressor genes
c). Both a and b
d). None of the above

9. The advantages of Northern blot are .....

a). Detection of RNA size
b). Quality and quantity of RNA can be measured
c). Use of probes with partial homology
d). All the above

10. Immunoblot is the other name of .....

a). Northern blot
b). Southern blot
c). Western blot
d). None of the above

11. Western blot is a method used to check for the presence of.....

a). DNA
b). RNA
c). Protein
d). None of the above

12. Southern blot was invented by .....

a). Edwin Southern
b). James Alwine
c). David Kemp
d). Neal Burnette

13. Eastern blot is the extension of .....

a). Northern blot
b). Southern blot
c). Western blot
d). None of the above

14. Eastern blot is a method used to check for the presence of.....

a). DNA
b). RNA
c). Protein
d). Protein post translational modification

15. Eastern blot was invented by .....

a). Edwin Southern
b). James Alwine
c). Thomas
d). Neal Burnette

16. The disadvantages of blotting techniques include .....

a). Complex, cumbersome and time consuming process

b). No information about regulation and gene interaction

c).Analysis of one gene or RNA at a time

d). All the above