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BCH 412

ASSIGNMENT

1- Explain the main steps involved in blotting procedures.

Blotting is a technique by which a macromolecules such as DNA, RNA, or protein is resolved in a gel matrix, transferred to a solid support and detected with a specific probe. Blotting procedures can be divided into six steps. There are 6 main steps.

a) Electrophoresis: The molecules of interest is present in a complex mixture of molecules. Electrophoresis (gel electrophoresis on either agarose for nucleic acid or polyacrylamide gel for protein) is used to separate the molecules resulting in the detection of protein or nuleic acid sequence of interest.

b) Transfer: After separation, the molecules (protein or nucleic acid) are transferred to a solid support such as nylon, nitrocellulose, or polyvinylidene fluoride (PVDF) membrane. This transfer allows the production of the replica of the molecules that were present in the gel which gets immobilized on a membrane. The same pattern of separation present on the gel must also be present on the membrane. The most common transfer techniques are capillary blotting, for use with southern or northern and electroblotting for immunoblots.

c) Blocking: This step helps to prevent nonspecific binding of the probe to the remaining binding sites on the membrane. Binding should be strictly to the molecules of interest. Prior to the addition of the

probe, measures are taken to avoid the nonspecific binding to the probe, measures such as treating the membrane with blocking agents (proteins or detergent).

d) Probing: Here, there is incubation of the membrane and the specific probe that binds to the protein or nucleic acid sequence of interest. For Southern blotting the probe will consist of a complementary DNA sequence that will anneal to the target. The same for RNA sequence in the case of Northern blot. for detection, the nucleic acid is tagged or labelled radioactively enzymatically. In the case of immunoblotting, the pro is an antibody specific for a specific protein or epito

A secondary antibody conjugated to a reagent wi aid detection and is thus incubated with the blot. There will be high affinity between the primary antibody and complementary secondary antibody and th facilitates the generation of a specific signal. Upon th period of incubation, sequential washing with specific reagents (wash buffers), are used to remove unbound probe or non-specifically bound probe.

e) Detection: The detection step involves visualizing the bound probe. The nature of the probe determines the method of detection. An X-ray film/phospho-imaging device is used for quantification of the bound probe a radioactive probe. The blot will be exposed to the method of detection. An enzymatic method implies detection by means of adding appropriate substrate to the blot. This gives a resulting signal that can be quantified by colorimetric, fluorescent or chemiluminescent imaging.

(2)

(2) In a tabular form, Compare and Contast the main types of blotting techniques, Under the following heading. Electrophoresis, transfer (different membranes that can be used), blocking, probing, detection and blotting results.

Results and Analysis: Once the blot is developed, the resulting banding pattern can be analyzed. Analysis involves determining the amount and apparent molecular weight or size of the molecules on the blot and comparing the results to the predicted pattern and to determine the molecular weight of the molecules of interest, a standard curve of size versus migration distance is derived from the molecular weight markers.