OKANDEJI JASMINE OGHENEMARO
16/MHS01/175
BCH 412 ASSIGNMENT 3

1. Explain the main steps involved in blotting procedures

i Homogenization of the sample which involves the purification of DNA/RNA proteins which is performed after extraction from a variety of sources such as cells or tissue.

ii Digest the DNA with restriction enzymes into fragments which is not required for RNA (Northern blot).

iii Separation of the molecule of interest by an electrophoresis membrane generally on an agarose gel for DNA fragments. In the case of RNA samples they can be separated on an agarose gel in the presence of formaldehyde as the denaturing agent. This is necessary as formaldehyde confines secondary structures of RNA molecules.

iv Transferring the molecules (DNA/RNA fragments) to a nitro cellulosic membrane / nylon membrane from the gel.

v Prehybridization (Blocking): Washing of the nylon membrane with a prehybridization or blocking solution comprising salmon sperm DNA is required in order to block non-specific DNA interactions and also this helps in the reduction of background noise. As an alternative, there are some commercially available blocking buffers like Perfect Hyb™ Plus buffer in which there is no requirement of salmon sperm DNA for blocking purpose

vi For the preparation of probe fresh probe DNA labelled with ^{32}P alpha-labelled dCTP is prepared.

vii Hybridization or identification of the molecule which is achieved by incubating the blot with the specific labelled probe.

viii For the detection of the probe and the sequence of interest DNA/RNA the film is exposed to −80°C.

	SOUTHERN BLOTTING	NORTHERN BLOTTING	WESTERN BLOTTING
Electrophoresis	Used to separate DNA fragments by size	Used to observe size of fragments in RNA samples	Used to separate mixtures of proteins based on molecular wt.
Transfer	Nylon membranes are used	Nylon membranes are also used	PVDF and nitro-cellulose membranes
Blocking	Solution containing Salmon Sperm DNA is used	Solution containing Salmon Sperm DNA is also used	BSA or non fat dried milk diluted in TBST/PBST buffers
Probing	Probes are pieces of DNA or RNA that have been modified	Probes are composed with nucleic acids	Secondary probing is carried out with a modified antibody
Detection	Probe hybridization is used for detection	Detection is also carried out with hybridization probe	Chemiluminescence, colorimetric and fluorescence methods
Blotting results	Results can be used to locate particular genes in a genome	Results show specific RNA molecules among mixtures	Blotting results show size of proteins