

Southern, Northern and Western Blotting

Blotting

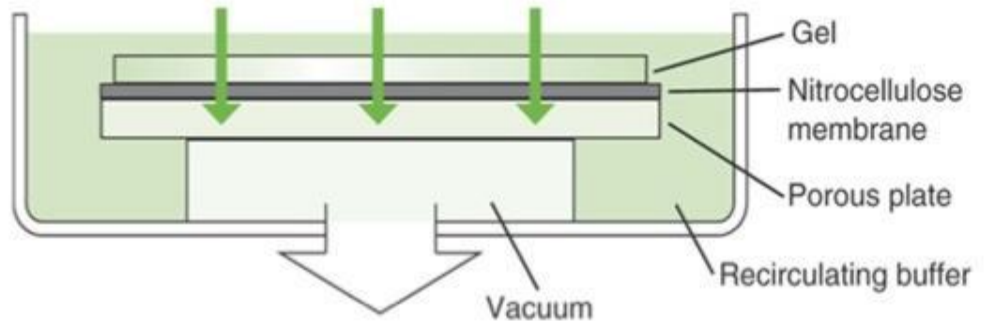
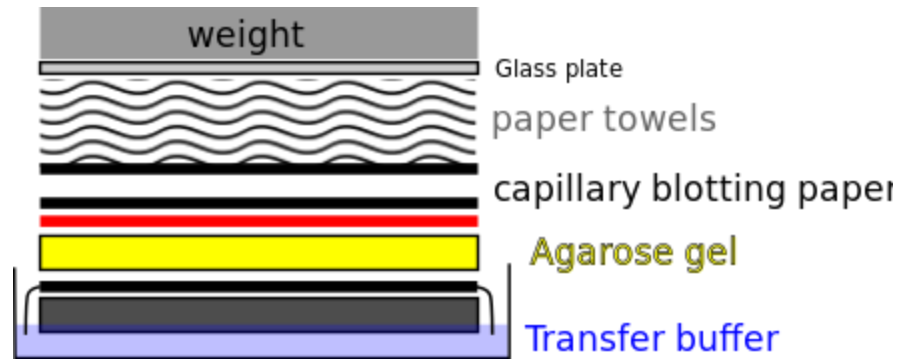
- It's the transfer of macromolecules like proteins and nucleic acids from gel onto surface of immobilised membrane similar to gel.
- These powerful techniques allow us to identify and characterize specific molecules in a complex mixture of related molecules

Blotting methods

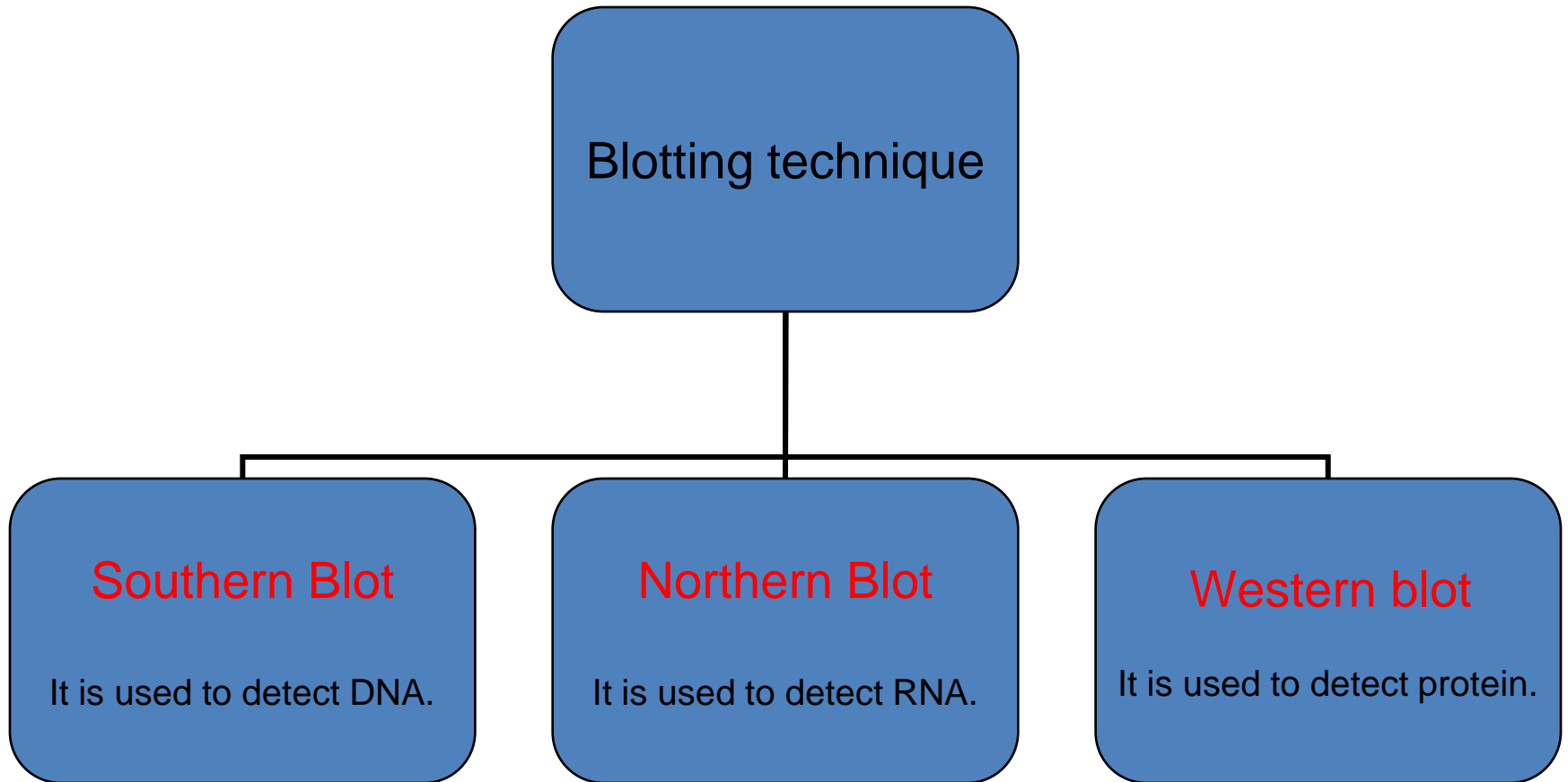
1. Diffusion blotting

2. Capillary blotting

3. Vacuum blotting



TYPES OF BLOTTING TECHNIQUES



Blotting membranes

- Nitrocellulose membrane
- Polyvinylidenedifluoride (PVDF) membrane
- Nylon membrane
- Diazobenzyloxymethyl (DBM) and
Diazophenylthioether (DPT)

Southern blotting



Ed Southern

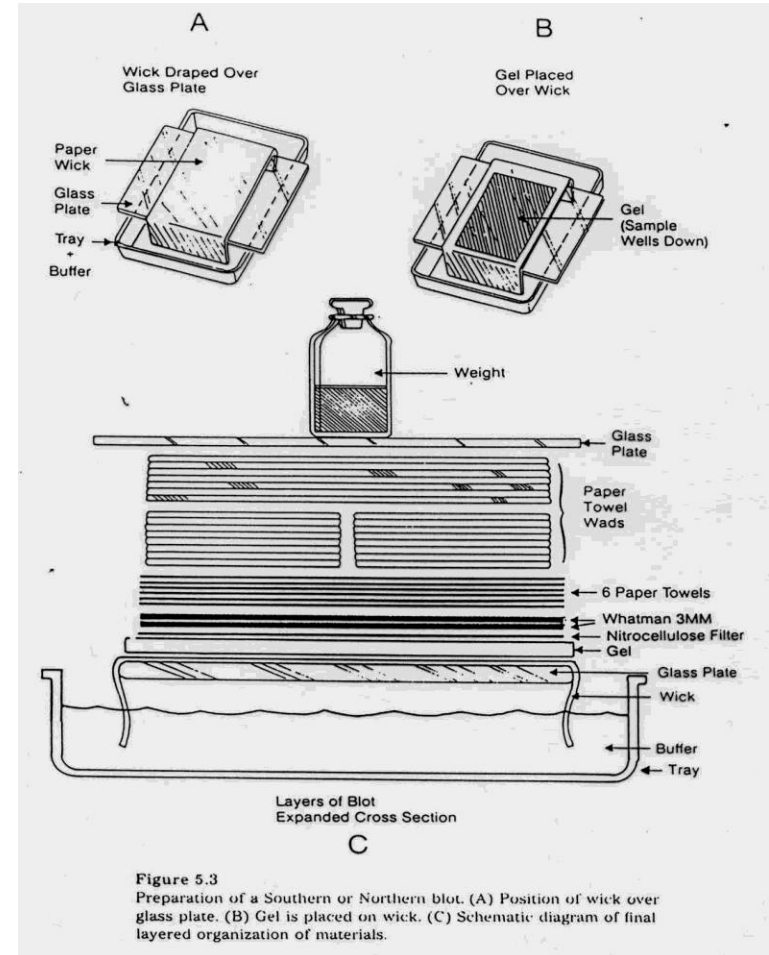
In the 1970s Ed Southern of Oxford University invented a revolutionary DNA blotting technique.

The Southern Blot allows the visualization of one DNA fragment from a whole genome DNA extract.

Southern Blot: DNA-DNA*

DNA-DNA hybridization

Uses gel electrophoresis together with hybridization probes to characterize restriction fragments of genomic DNA (or DNA from other sources, such as plasmids)



Steps

Digest DNA with restriction endonuclease



Electrophoresis to separate fragments



Blot fragments onto a nitrocellulose membrane

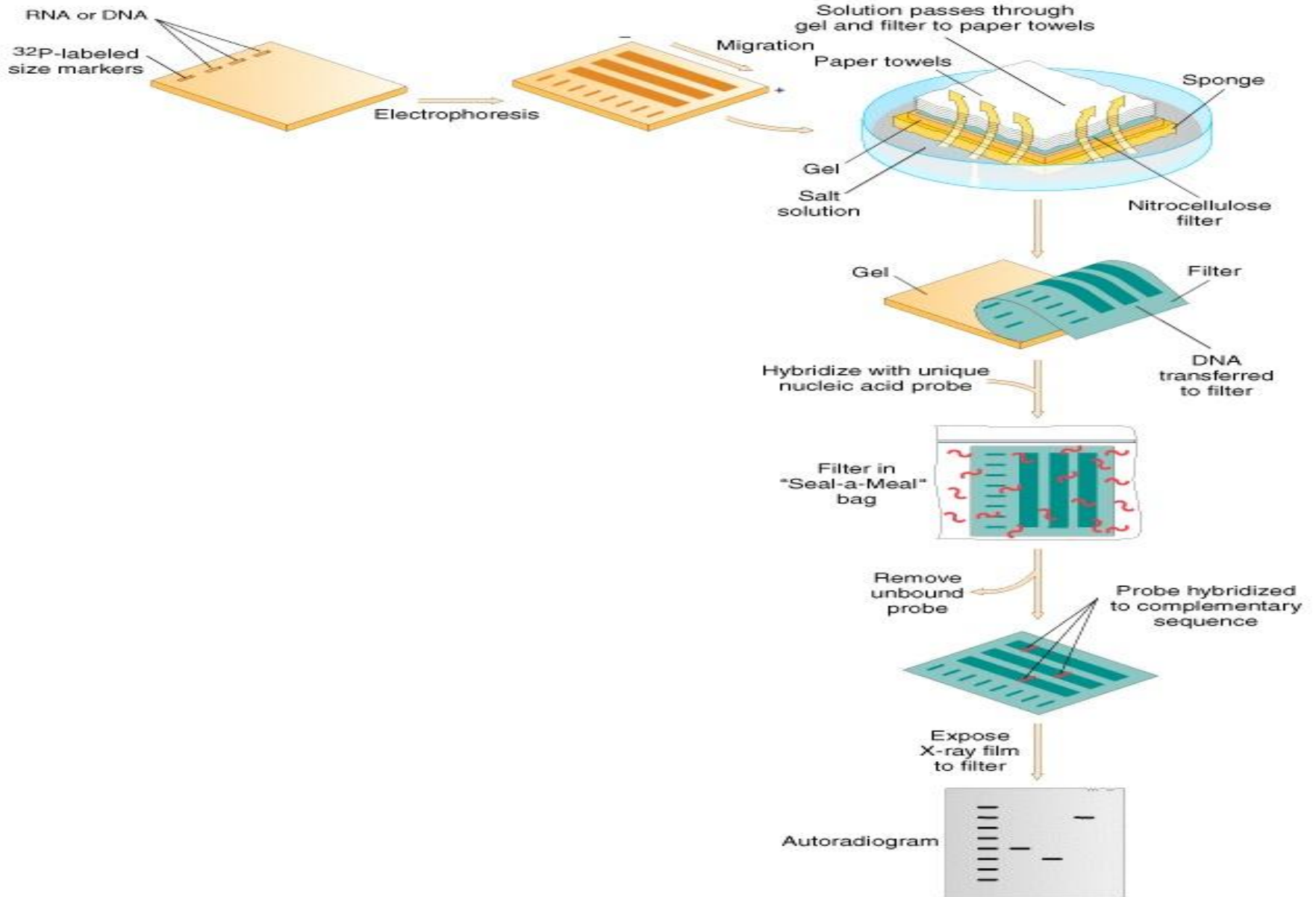


Hybridize with the ^{32}P probe



Autoradiography

General Scheme for Southern Blot



Step 2

Gel electrophoresis

- Separates DNA fragments.

Soak gel in 0.5 M NaOH

- Converts dsDNA to ssDNA



Step 3. Nitrocellulose Blot

Cover gel with nitrocellulose paper

paper



Cover nitrocellulose paper with thick layer of paper towels



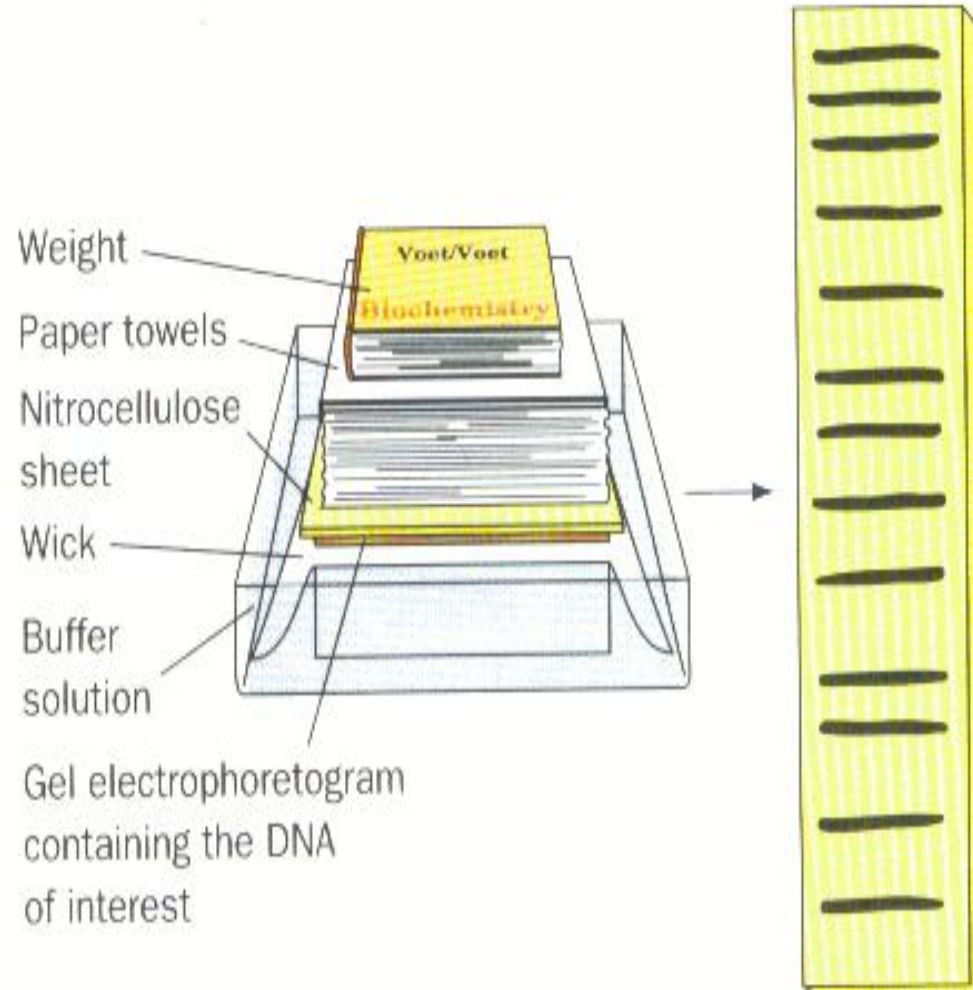
Compress apparatus with heavy weight



ssDNA binds to nitrocellulose at same position as on the gel



Vacuum dry nitrocellulose at 80°C to permanently fix DNA in place or cross link (via covalent bonds) the DNA to the membrane



Step 4. Hybridization

- Incubate nitrocellulose sheet with a minimal quantity of solution containing ^{32}P -labeled ssDNA probe.
- Probe sequence is complementary to the DNA of interest.
- Incubate for several hours so that probe can anneal to its target sequence(s).
- Wash & dry nitrocellulose sheet.

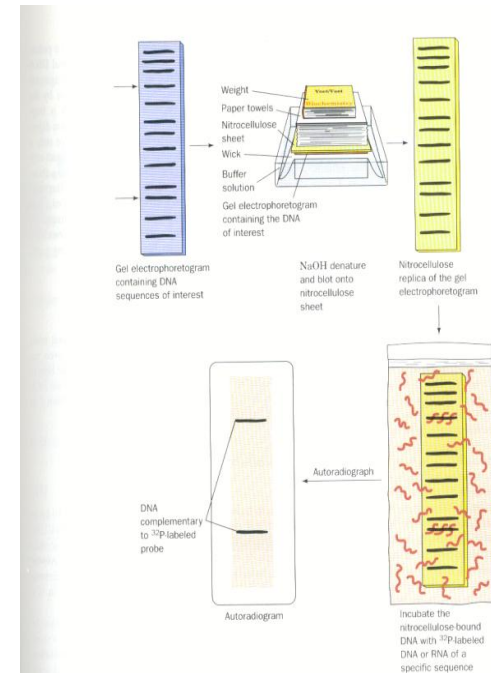


FIGURE 5-50 Detection of DNAs containing specific base sequences by the Southern transfer technique.

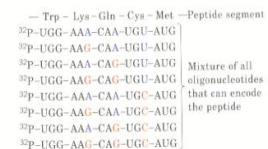
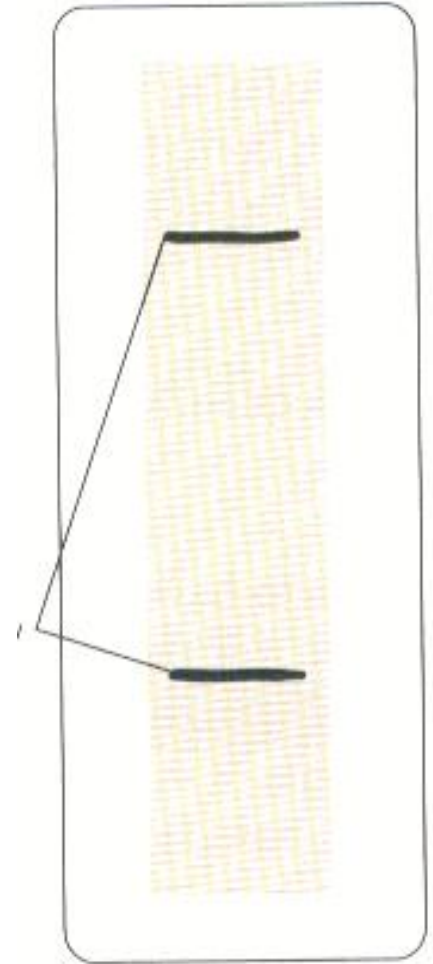


FIGURE 5-51 A degenerate oligonucleotide probe. Such a probe is a mixture of all oligonucleotides that can encode a polypeptide segment of known sequence. In practice, such a segment is chosen to contain a high proportion of residues specified by low-degeneracy codons. In the pentapeptide segment shown here, Trp and Met are each specified by only one codon and Lys, Gln, and Cys are each specified by two codons that differ in only their third positions (blue and red; Table 5-3) for a total of $1 \times 2 \times 2 \times 2 \times 1 = 8$ oligonucleotides. The oligonucleotides are ^{32}P -labeled for use in Southern blotting.

Step 5. Autoradiography

- Place nitrocellulose sheet over X-ray film.
- X-ray film darkens where the fragments are complementary to the radioactive probes.



Application

1. Diagnosis & detection of genetic diseases

- Used to diagnose sickle cell-anemia.
- A→T base change in the β subunit of Hb, Glu→ Val
- Development of a 19 residue oligonucleotide probe complementary to sickle-cell gene's mutated segment.

- Probe hybridizes to DNA from homozygotes of sickle-cell anemia but not from normal individuals.
- Used in gene discovery , mapping, evolution and development studies, diagnostics and forensics (DNA fingerprinting, preparation of RFLP maps)
- Identification of the transferred genes in transgenic individuals

- To determine the molecular weight of a restriction fragment and to measure relative amounts in different samples.
- Analyze the genetic patterns which appear in a person's DNA.

Northern Blot: RNA-DNA*(RNA*)

- technique was developed in 1977 by **James Alwine, David Kemp, and George Stark** at **Stanford University**.
- No need to digest RNA with REs.
- SS RNA can form the secondary structure during running.
- **Use formaldehyde to break H-bonds** and denature RNA because **single-stranded RNA** will form intramolecular base pairs and "fold" on itself.

Steps

Isolate RNA & treat with formaldehyde



Agarose gel electrophoresis



Transfer single-stranded RNA to nitrocellulose or nylon membrane



Incubate membrane with labeled DNA or RNA probe



Autoradiography

Step 1

Isolate RNA:

To detect rare mRNA, isolate the poly A⁺ mRNA.

RNA is both biologically and chemically more labile than DNA. Thus eliminate RNases.

Step 2

Electrophoresis:

Performed in formaldehyde agarose gel to prevent RNA from folding on itself.

Stain with EtBr to visualize the RNA bands.

PAGE can be used with Urea.

Step 3

- Transfer ss RNA to nitrocellulose or nylon membrane:
- **Transfer buffer contain formamide (it lowers the annealing temperature of the probe-RNA interaction)**

Nitrocellulose typically has a binding capacity is lesser than nylon

- Covalently link RNA to membrane:

UV cross linking is more effective in binding RNA to the membrane than baking at 80°C.

Step 4 & 5

- Prehybridize before hybridization:

Blocks non-specific sites to prevent the single-stranded probe from binding just anywhere on the membrane.

- Incubate membrane with labeled DNA or RNA probe with target sequence:

Probe could be ^{32}P , biotin/streptavidin or a bioluminescent probe.

- Autoradiography:

Place membrane over X-ray film.

X-ray film darkens where the fragments are complementary to the radioactive probes.

Application

- To determine the conditions under which specific genes are being expressed (mRNA level).
- A standard for the study of gene expression at the level of mRNA (messenger RNA transcripts)
- Detection of mRNA transcript size

- Study RNA degradation
- Study RNA splicing
- Often used to confirm and check transgenic/
knockout mice (animals)

Southern vs. Northern

Southern

- DNA on membrane
- Digest DNA
- Convert dsDNA to ssDNA

Northern

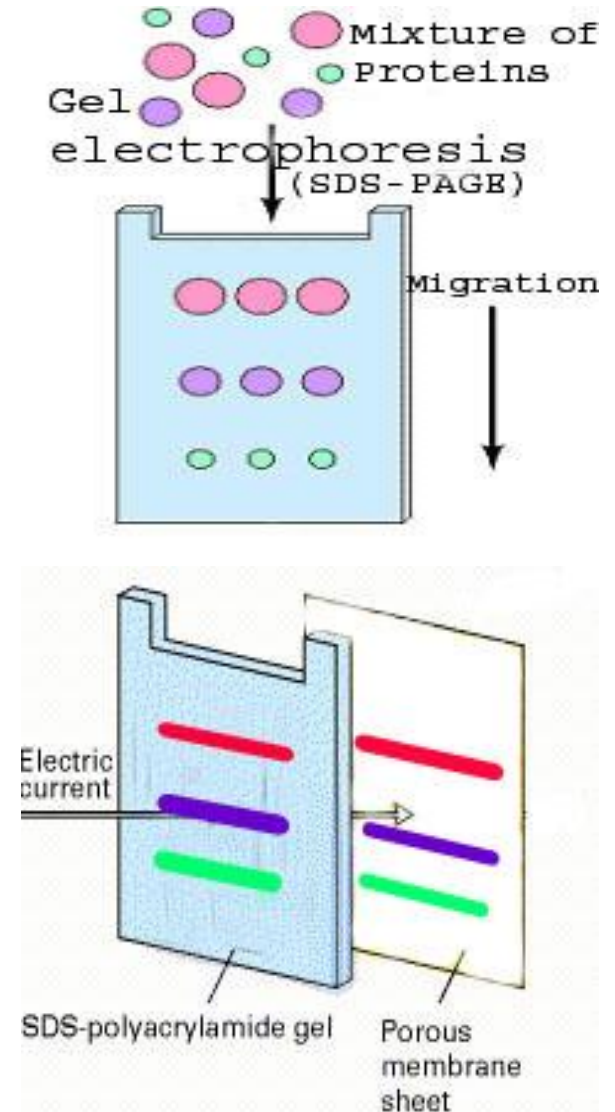
- RNA on membrane
- No need to digest DNA
- Denature “folded” RNA with formaldehyde

Western blotting

- An Immunoblotting technique based on the specificity of binding between a protein of interest and a probe to allow detection of the protein of interest .
- The SDS PAGE technique is a prerequisite for Western blotting .

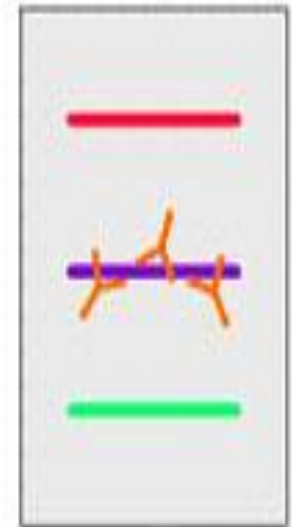
Steps in western blotting

1. A protein sample is subjected to electrophoresed on an SDS-polyacrylamide gel.
2. Electroblotting transfers the separated proteins from the gel to the surface of a nitrocellulose membrane.

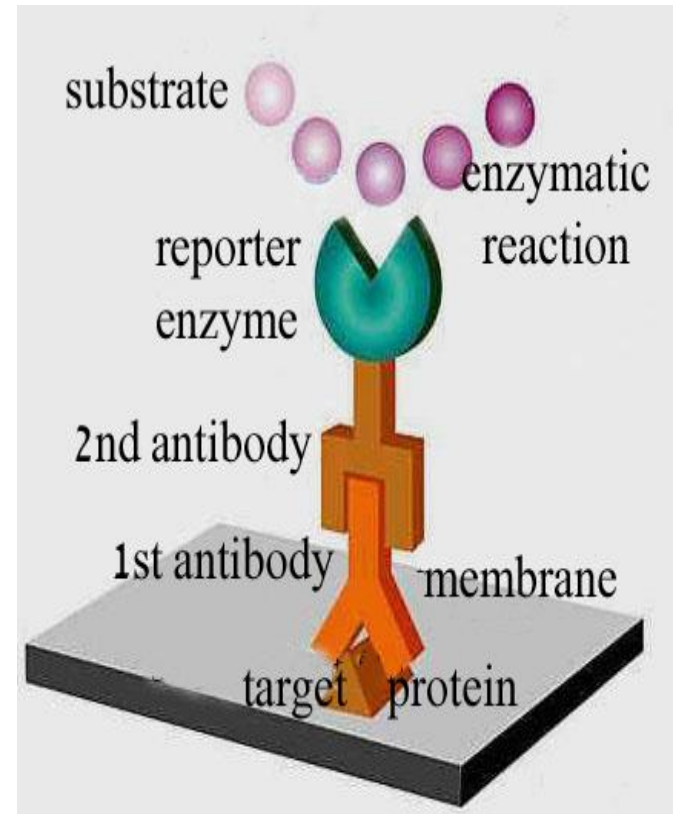


3. The blot is incubated with a generic protein (such as milk proteins or BSA) which binds to any remaining sticky places on the nitrocellulose.
4. An antibody that is specific for the protein of interest (the primary antibody - Ab1) is added to the nitrocellulose sheet and reacts with the antigen.

 - Only the band containing the protein of interest binds the antibody, forming a layer of antibody molecules .



5. After washing for removal of non-specifically bound Ab1, second antibody (Ab2) is added.
- Ab2 specifically recognizes the primary antibody and binds.
 - Ab2 is radioactively labeled, or is covalently linked to a reporter enzyme, which allows to visualize the protein-Ab1-Ab2 complex.



Comparison of Southern, Northern, and Western blotting techniques

	Southern blotting	Northern blotting	Western blotting
Molecule detected	DNA (ds)	mRNA (ss)	Protein
Gel electrophoresis	Agarose gel	Formaldehyde agarose gel	Polyacrylamide gel
Gel pretreatment	Depurination, denaturation, and neutralization	-	-
Blotting method	Capillary transfer	Capillary transfer	Electric transfer
Probes	DNA Radioactive or nonradioactive	cDNA, cRNA Radioactive or nonradioactive	primary antibody
Detection system	Autoradiography Chemiluminescent Colorimetric	Autoradiography Chemiluminescent Colorimetric	Chemiluminescent Colorimetric

Application

1. The confirmatory HIV test
2. Used as the definitive test for Bovine spongiform encephalopathy (BSE)
3. Some forms of Lyme disease testing employ Western blotting.