# Blotting Methods

- **Southern blotting** involves the transfer of DNA from a gel to a membrane, followed by detection of specific sequences by hybridization with a labeled probe.
- **Northern blotting**, RNA is run on a gel.
- **Western blotting** entails separation of proteins on an SDS gel, transfer to a nitrocellulose membrane, and detection proteins of interest using antibodies.

# SOUTHERN BLOTTING

# SOUTHERN BLOTTING

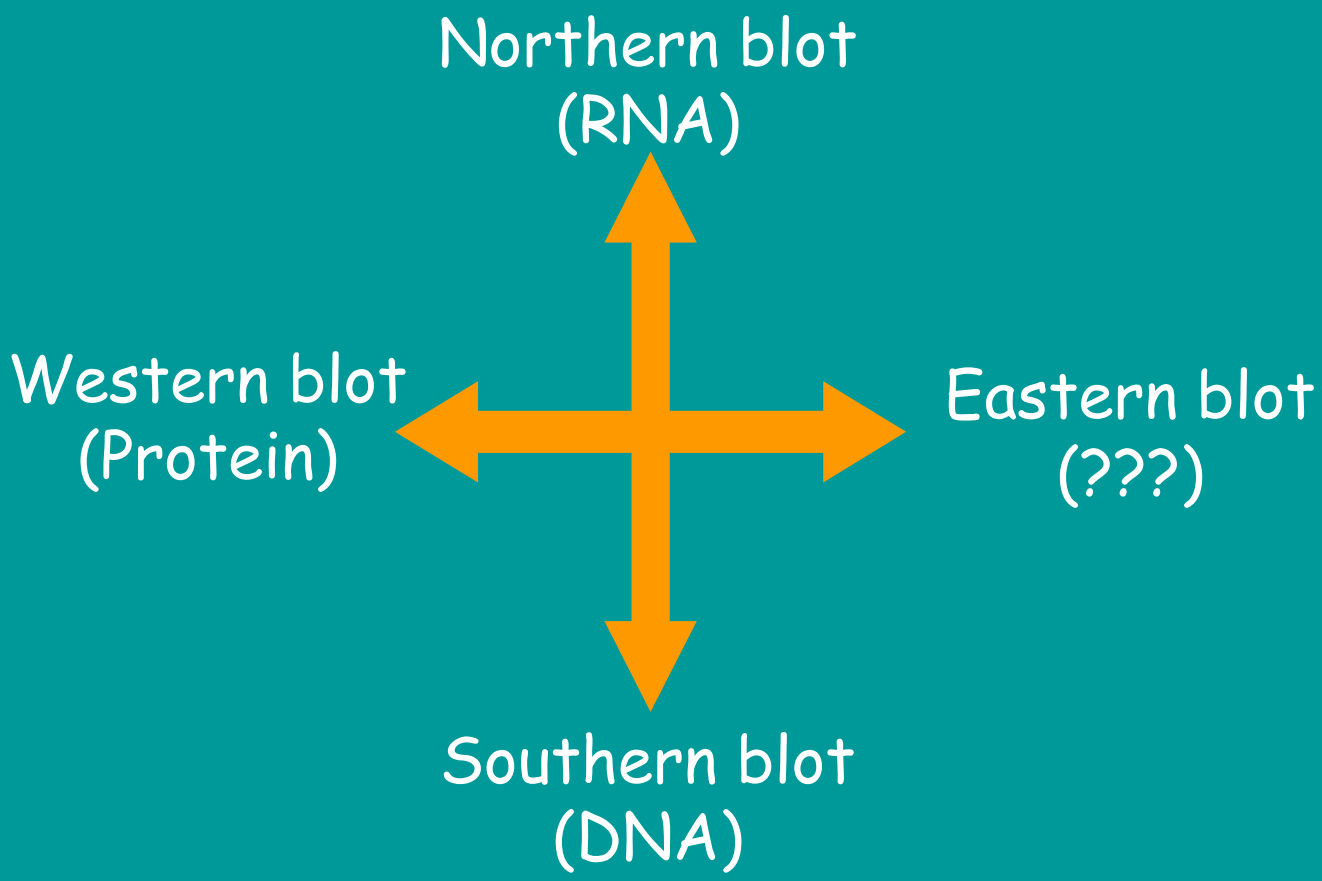
- The technique was developed by E.M. Southern in 1975.
- The Southern blot is used to detect the presence of a particular piece of DNA in a sample.
- The DNA detected can be a single gene, or it can be part of a larger piece of DNA such as a viral genome.

# OUTLINE

- DNA
- SPECIMEN COLLECTION AND STORAGE
- PROCEDURE
- WATCHPOINTS
- USES

# History/Background

- Spawned naming of related techniques:



# DNA

- The deoxyribonucleic acid, DNA, is a long chain of nucleotides which consist of:
- 1. Deoxyribose(sugar with 5 carbons)
- 2. Phosphate groups
- 3. Organic(nitrogenous)bases

# What are Southern and Northern Blots?

- A southern blot is a method used to detect specific DNA sequences in complex DNA samples.
  - It is a combination of several molecular biology techniques:
    - Restriction enzyme analysis
    - Agarose gel electrophoresis
    - Hybridization analysis
  - After electrophoresis, DNA molecules are transferred from the agarose gel onto a filter membrane for probe hybridization.
- A northern blot is almost identical to a Southern blot, but it involves the detection of RNA instead of DNA.

# History of the Southern blot

- The Southern blot technique was devised in 1975 by Edwin Southern at Edinburgh University in Scotland.
- He noticed how porous agarose gels were and realized that this property could be utilized to transfer the DNA to a more easily manipulated filter membrane.
  - Agarose gel is quite fragile and difficult to work with.
- For the development of this technique, Southern was awarded the Lancaster Award for Clinical Medical Research in 2005.

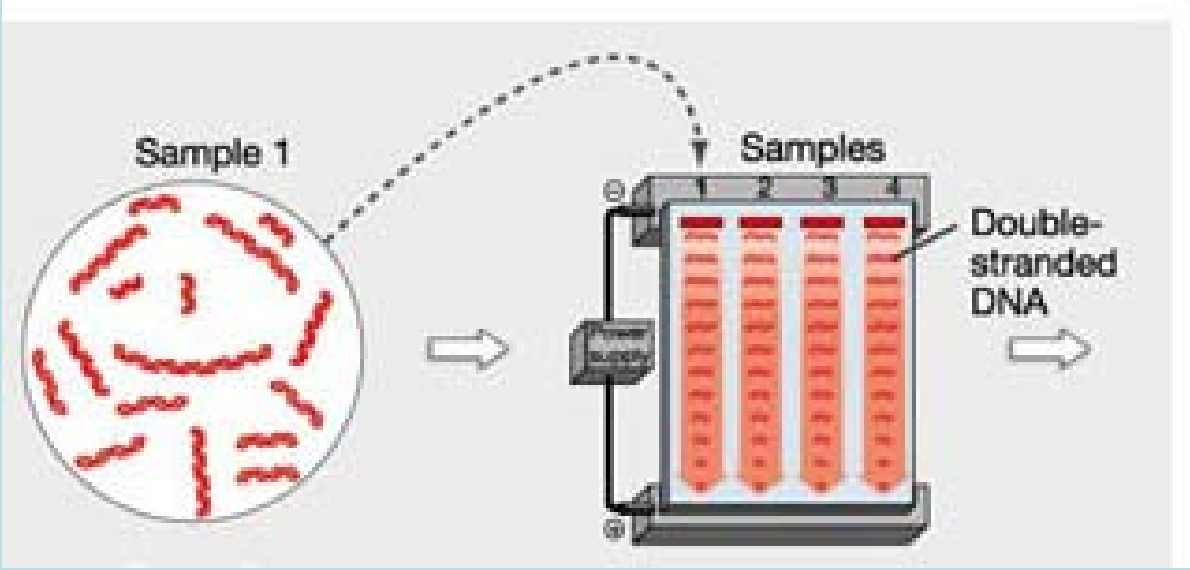
# Southern Blot Procedure

## Digest the dsDNA

- The sample DNA is isolated and digested with restriction endoonucleases.
- This results in the production of dsDNA fragments of varying length depending on the location of the restriction sites.

# Southern Blot Procedure

- Step 2: Run the digest.
  - The resulting restriction fragments are separated through agarose gel electrophoresis.
    - This separation is based on fragment size.

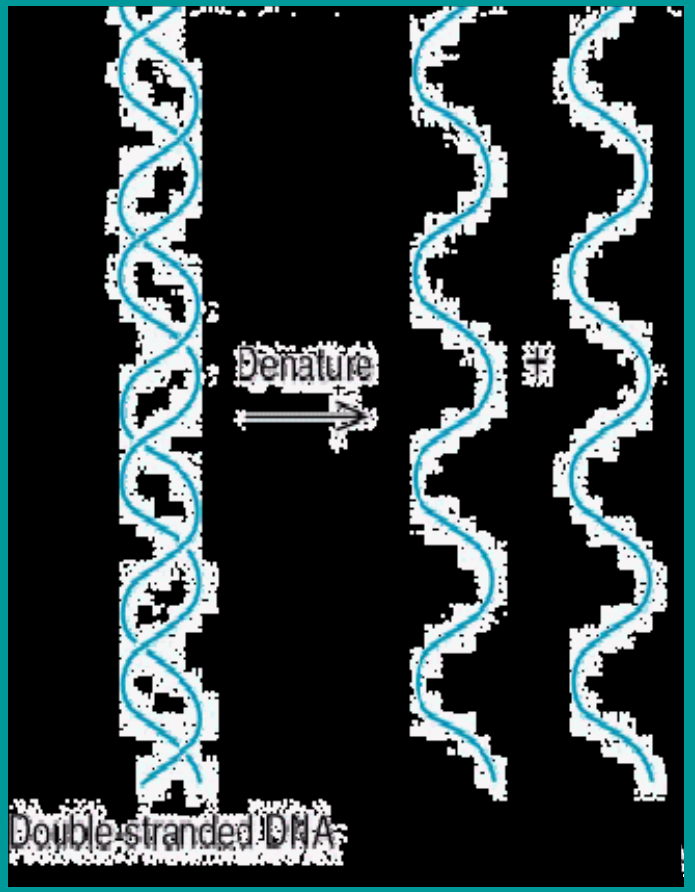


# Southern Blot Procedure

- Step 2 Continued: Run the digest.
  - Standard DNA size markers should also be run so the size of the sample fragments can be estimated.
  - Stain with ethidium bromide so that the DNA can be visualized under UV light.
    - Photograph the agarose gel alongside a ruler so that the distance from the wells to the DNA bands can be determined.

# Southern Blot Procedure

- Step 3: Denature the DNA
  - The agarose gel is soaked in a basic solution, such as NaOH.
  - After this step, it is important to neutralize the base before proceeding to the membrane transfer step.



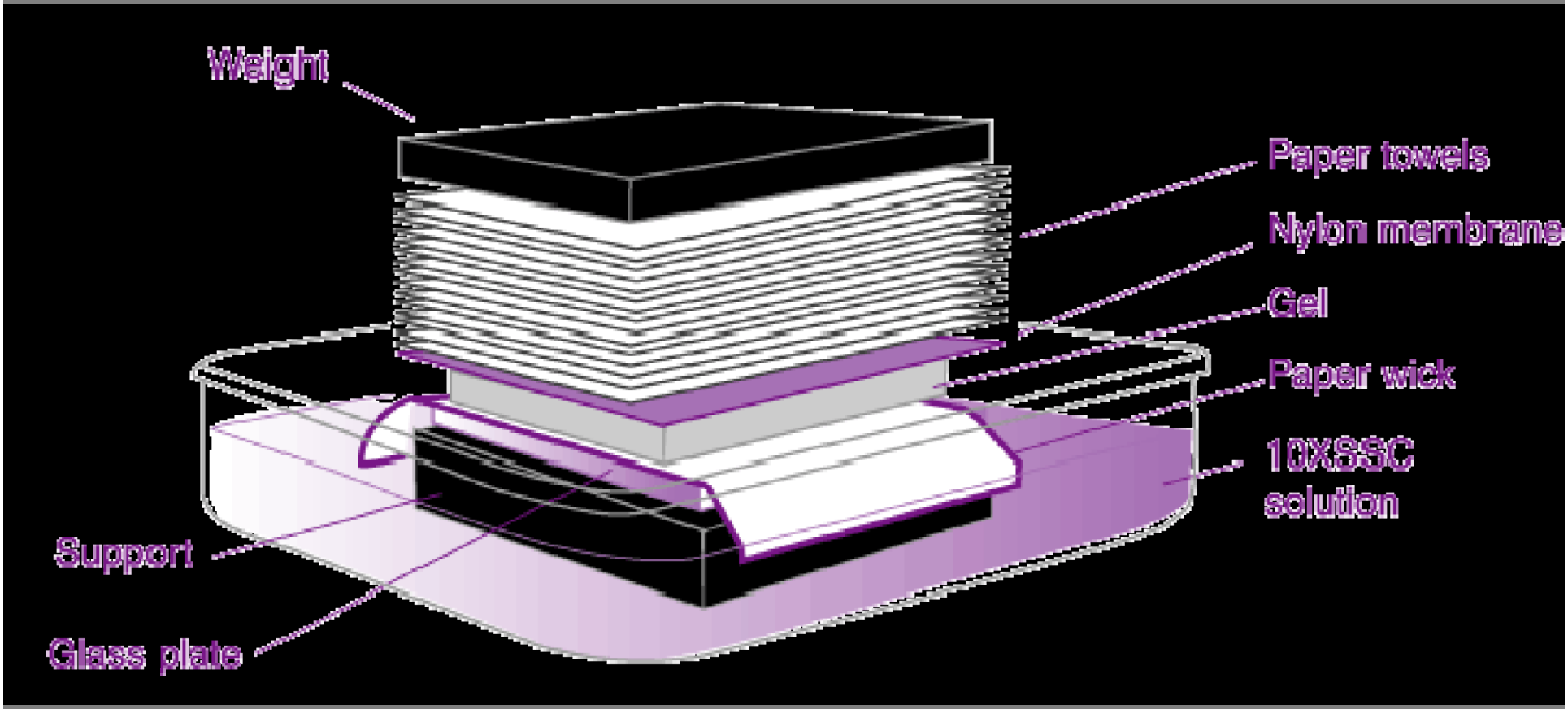
# Southern Blot Procedure

- A depurination step is optional.
  - DNA fragments that are larger than 15kb may be difficult to transfer to the filter membrane.
  - HCl can be used to remove the purines, thus reducing the size of the fragments.
  - It's important to note that the procedure may be hampered if the fragments are too small.

# Southern Blot Procedure

- Step 4: Transfer the denatured DNA to a filter membrane.
  - Nitrocellulose or nylon filter membranes may be used.
    - Nylon typically has a higher binding capacity and is less fragile.
  - In the Southern blot procedure, the DNA fragment transfer is achieved by capillary action.
    - Electrotransfer may also be used but is not as common.
  - After the transfer, the filter membrane should be exposed to UV light to cross link the DNA fragments to the membrane.

# Southern Blot Transfer Method



# SOUTHERN BLOTTING

- 2) Capillary blotting-fragments are eluted from the gel and deposited onto the membrane by buffer that is drawn through the gel by capillary action.

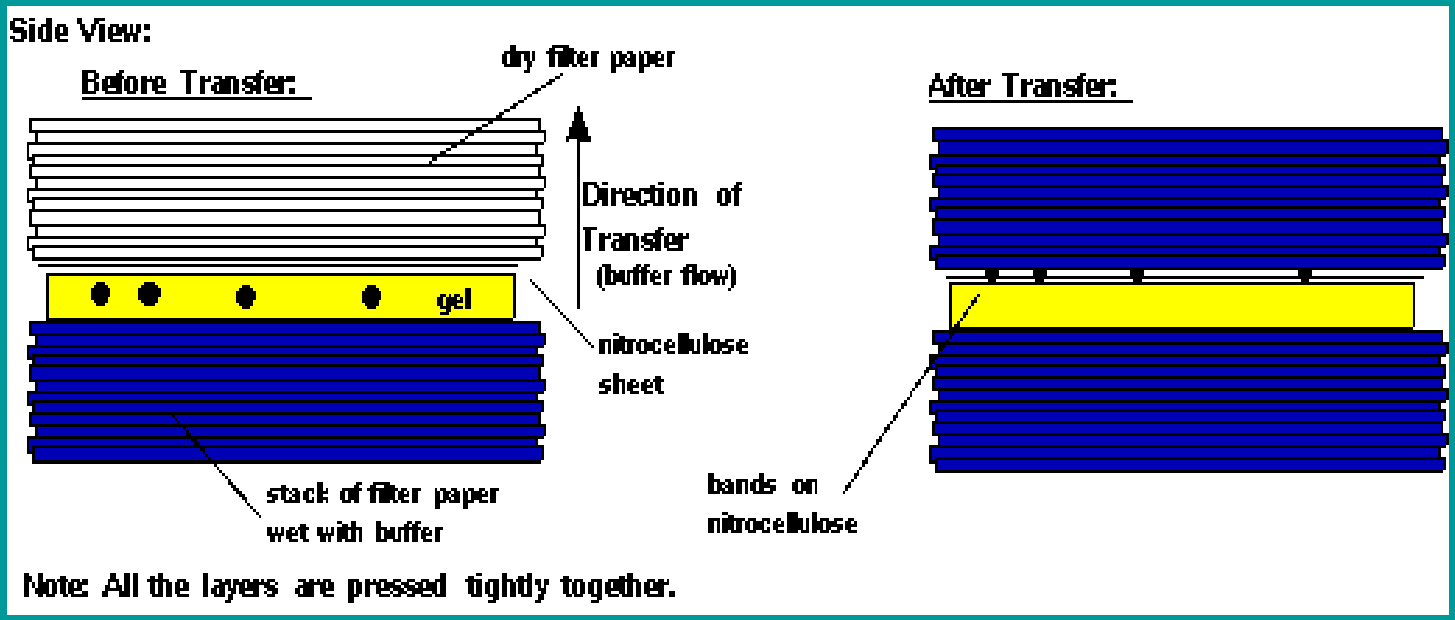
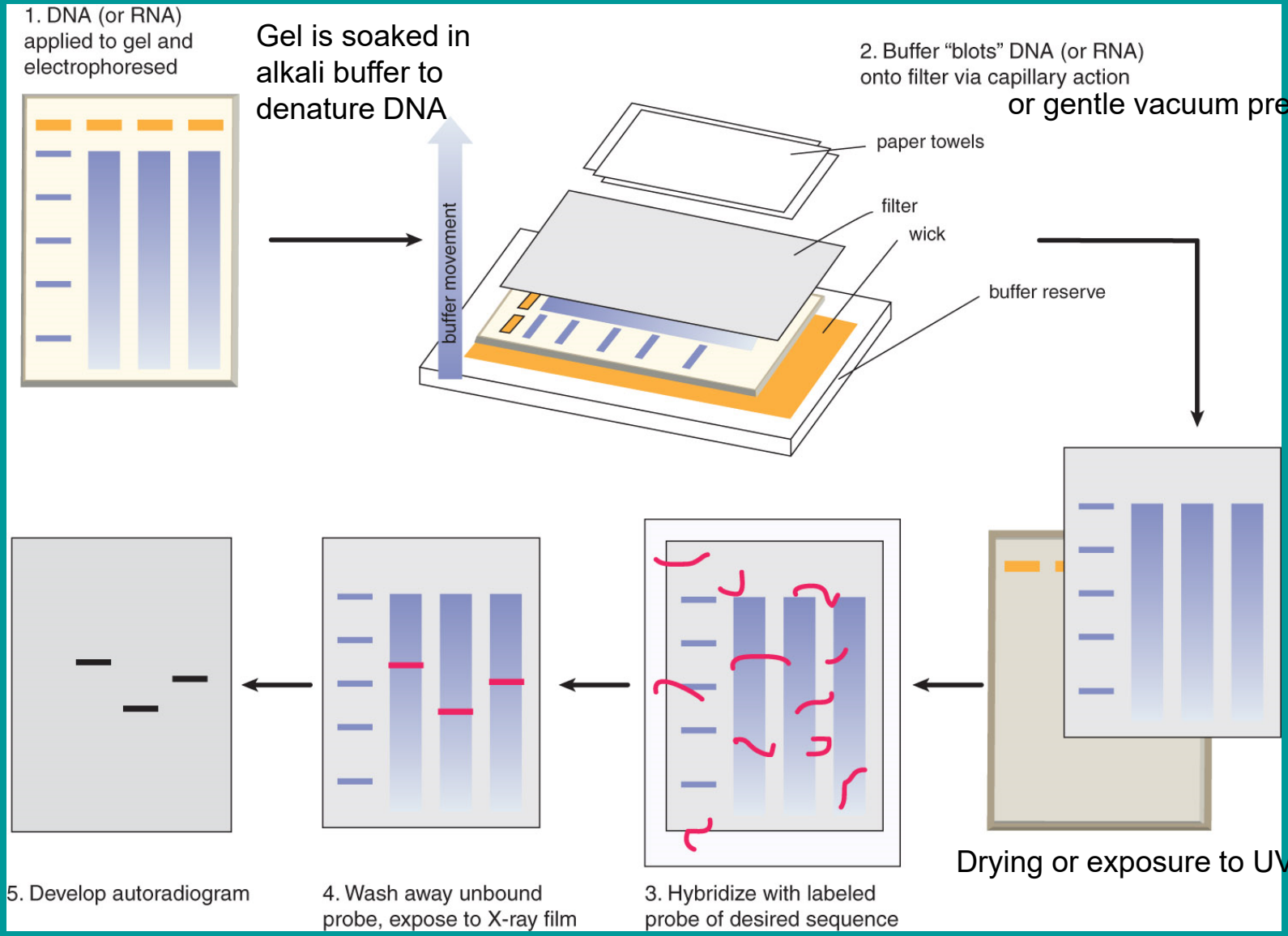


FIGURE 21: **Southern blot**: Identifying Specific DNA Fragments
(Edward Southern--the pioneer)



# Southern Blot Procedure

- Step 5: Hybridization Analysis

  A) The membrane is soaked in a prehybridization buffer.
  - This prevents the any nonspecific binding of the probe to the membrane

  B) The filter membrane is incubated with many copies of a ssDNA probe under specific conditions.
  - This probe contains a sequence that is complementary to the DNA sequence of interest.
  - If the sequence of interest is present on the membrane, the probe will anneal to this sequence.

# Hybridization Analysis Continued

C) The membrane is washed to remove any probe that has not hybridized.

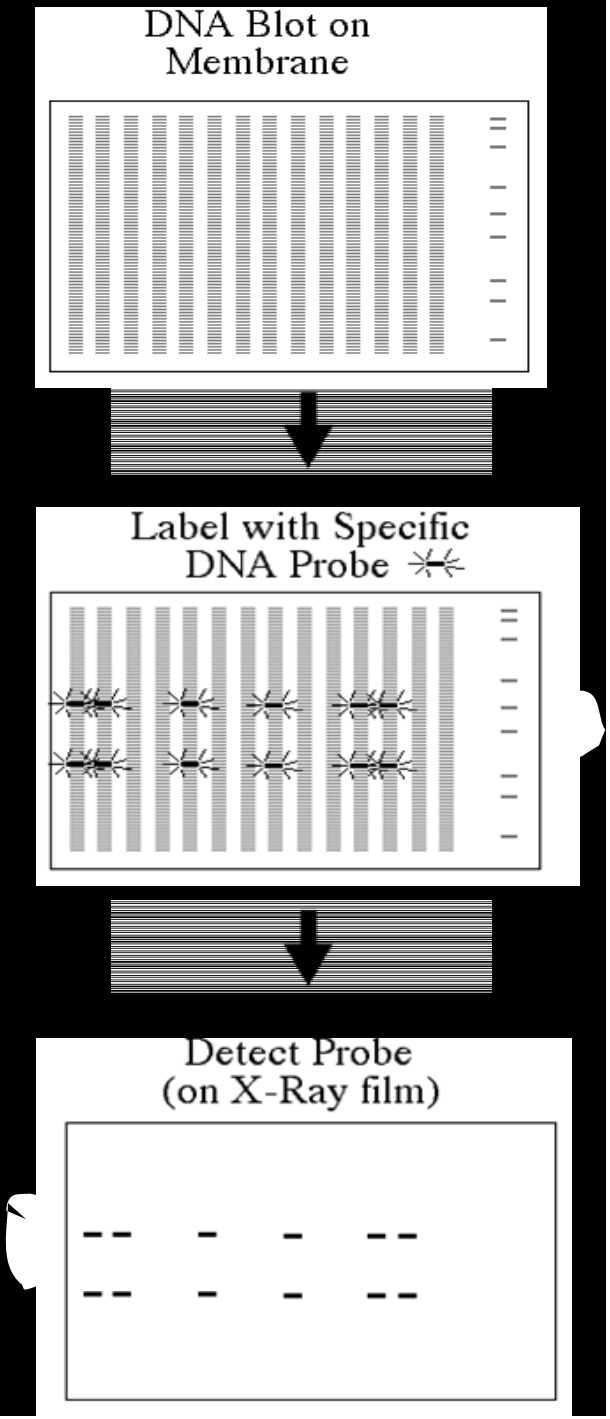
- Specific conditions needed that will remove any nonspecifically bound probe, but will not disrupt the probe-target complex.

D) The membrane is tested for the presence of any hybridized probe.

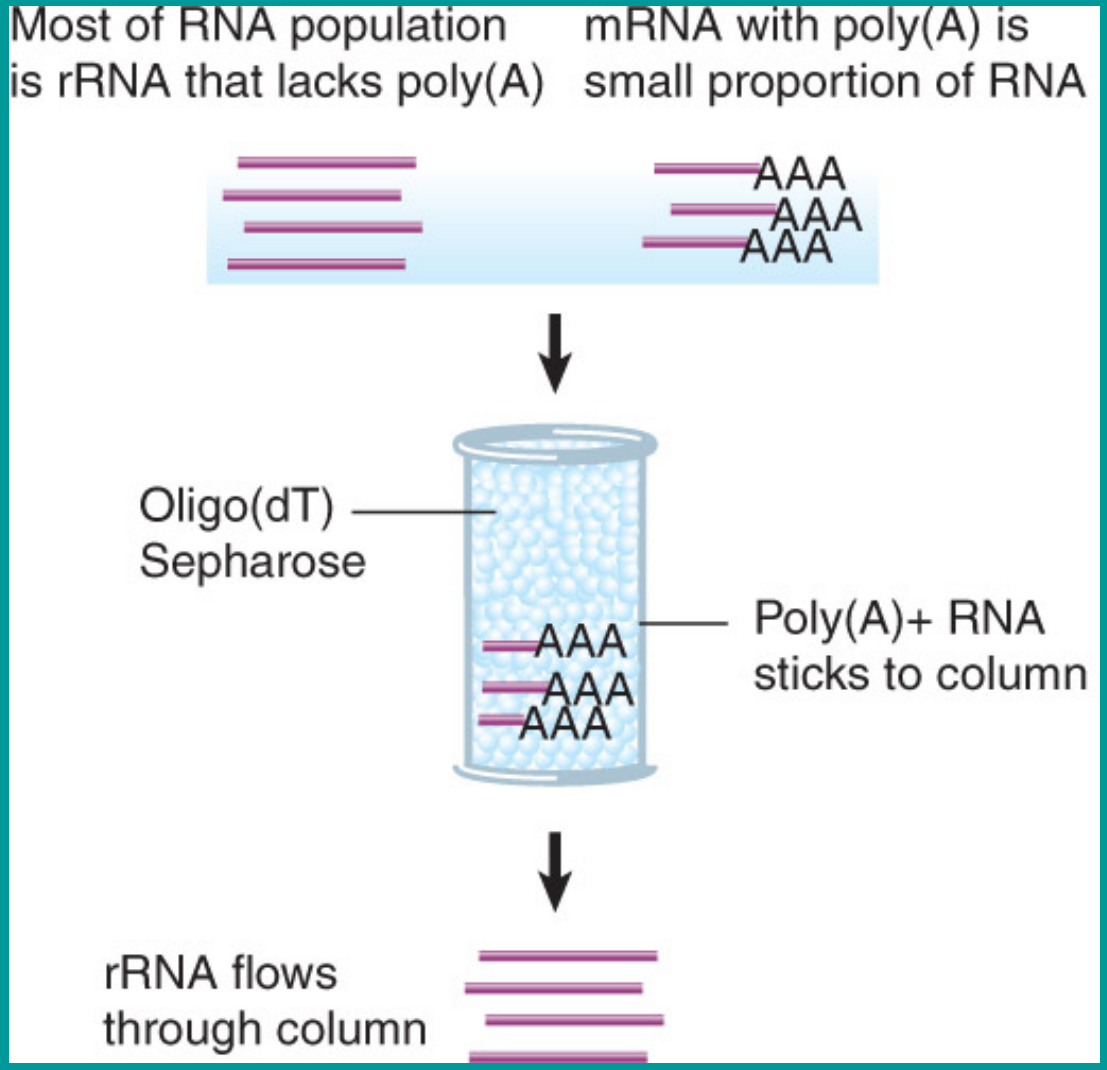
- Probes are either:
  - Enzyme labeled
  - Radioactively labeled

# Probe Detection

- Radioactively labeled probes:
  - Detected by exposing the membrane to X-ray film.
    - Autoradiography
    - Ares were hybridization has occurred will appear as a dark spot on the autoradiogram.
  - Probe usually labeled with $^{32}P$
    - High energy β-particle emitter.

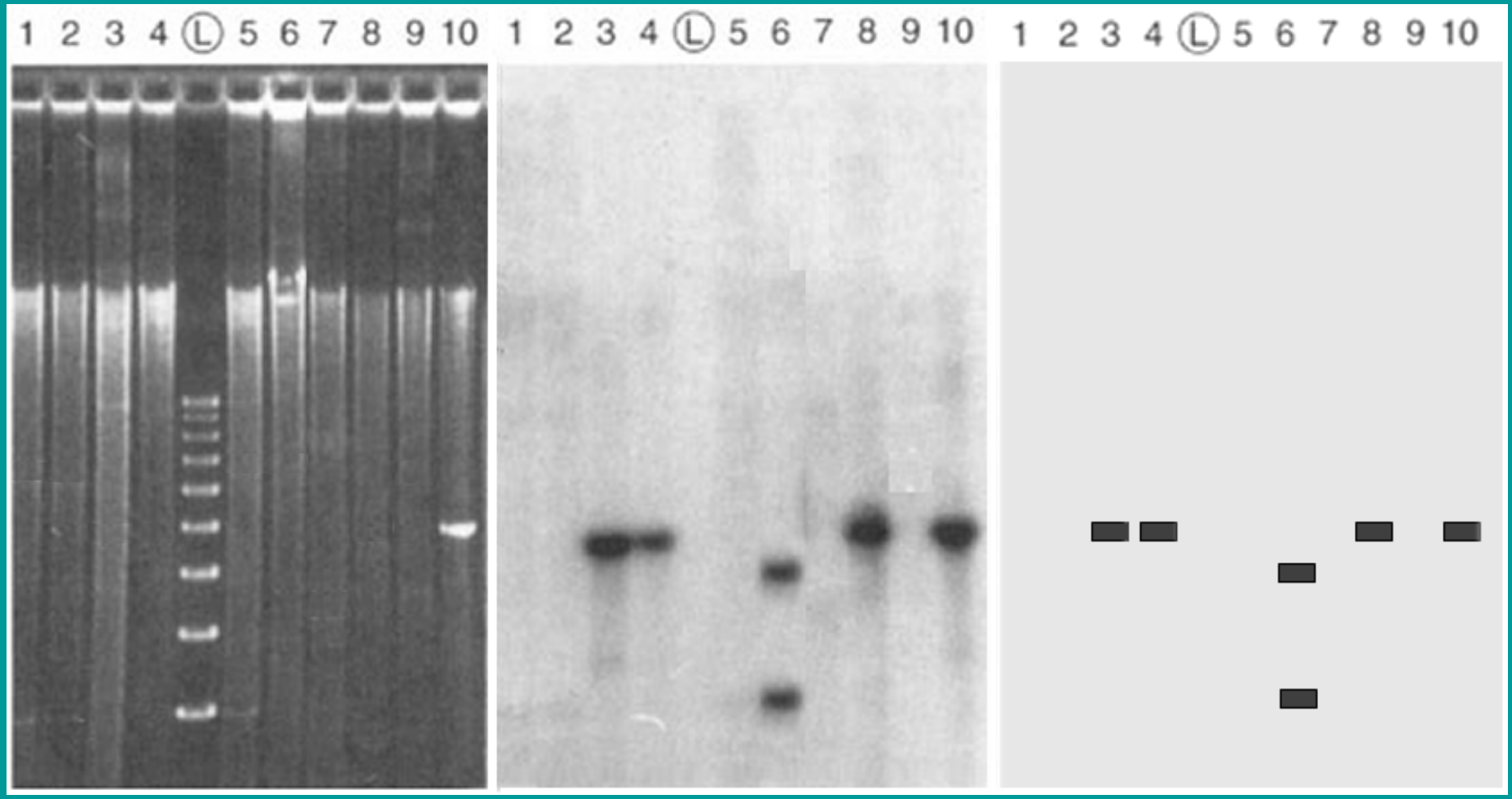


# How to separate mRNA from all other classes of RNA




mRNA contains ~200 oligo(A) residues at 3' end

FIGURE 22: Poly(A)+ RNA can be separated from other RNAs by fractionation on an oligo(dT) column

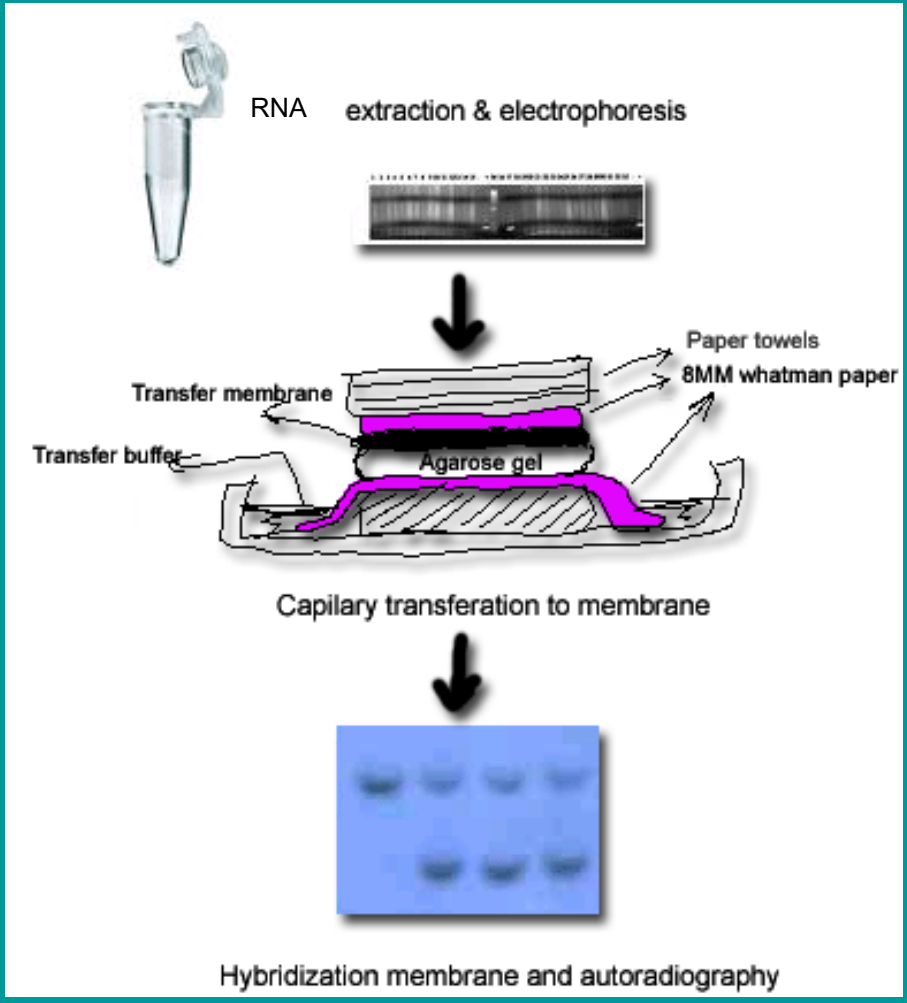



- The left panel shows an agarose gel after electrophoresis and staining with ethidium bromide.
- The center panel shows a Southern blot autoradiogram.
- The right panel shows a representation of the autoradiogram.

# USES

- Identify mutations, deletions, and gene rearrangements
- Used in prognosis of cancer and in prenatal diagnosis of genetic diseases
- Leukemias
- Diagnosis of HIV-1 and infectious disease

**Northern blotting** is similar to Southern blotting, but involves the transfer of RNA from a gel to a membrane



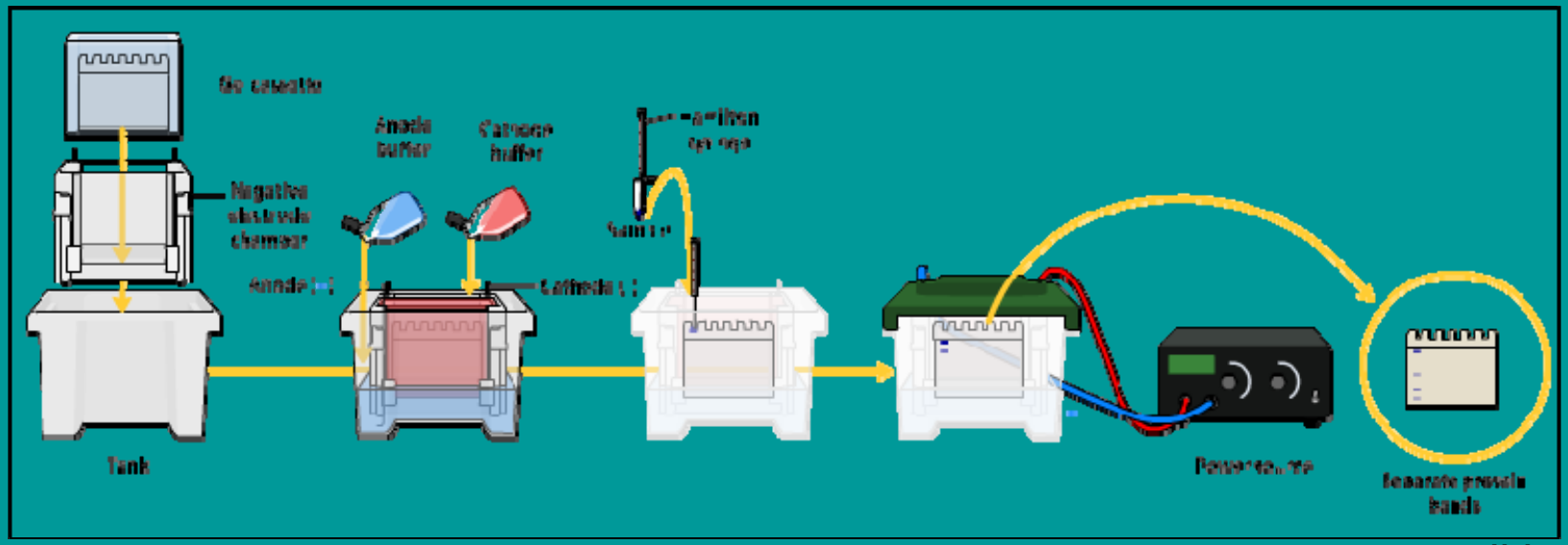
# Western Blotting

Protein analysis

- Western Blotting; is an immunoassay technique to assess the presence, amount, and molecular weight of proteins in cellular or tissue extracts by using antibodies.

# Western blotting

•**Western blotting** entails separation of proteins on an SDS gel, transfer to a nitrocellulose membrane, and detection proteins of interest using antibodies.



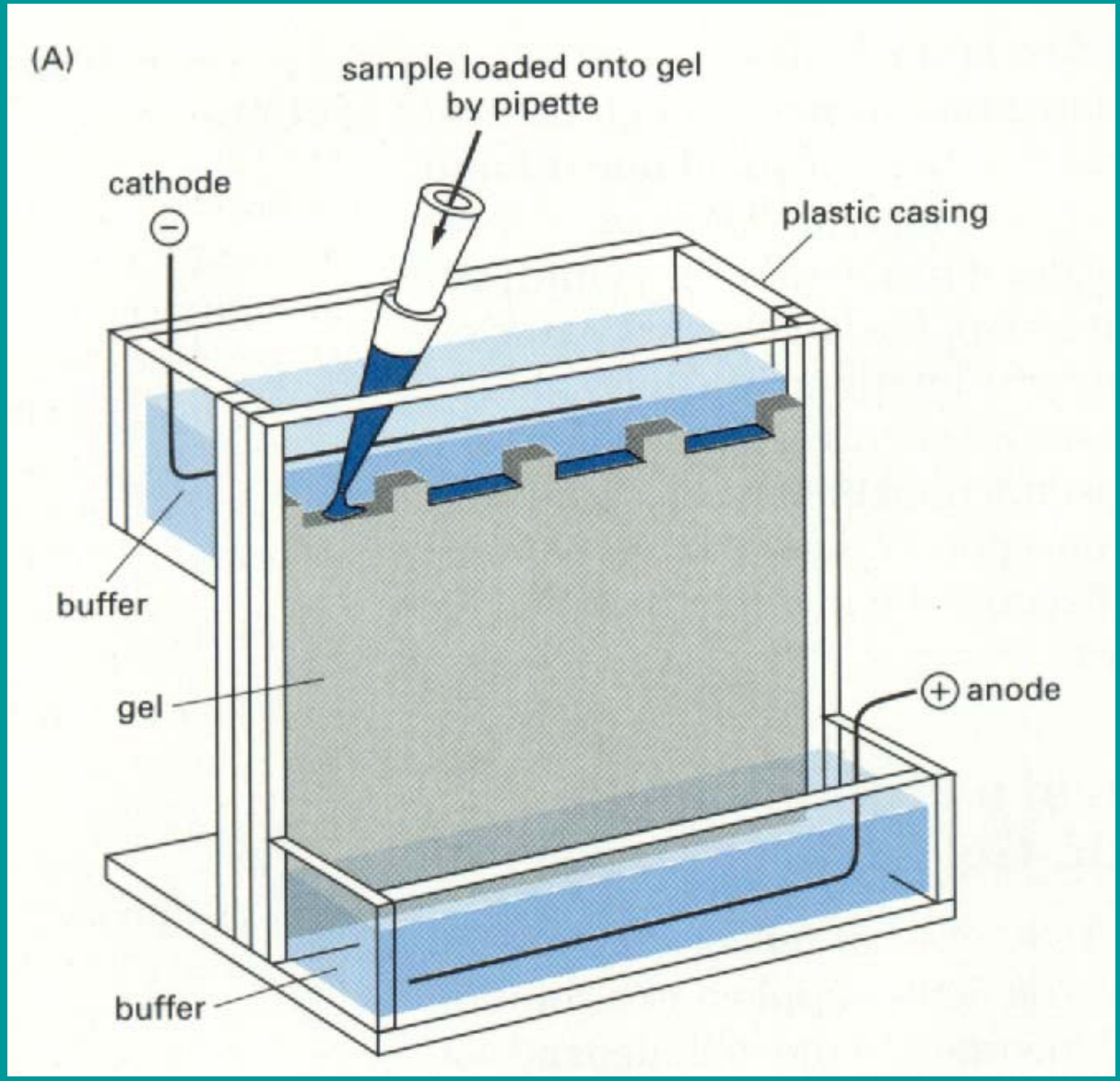

wikipedia

# Why is it called W.B.?

- It was called after the technique “Southern blotting”(as a joke) which uses the same approach  to detect DNA in cellular or tissue extracts. Southern blotting was first described by “Southern” in 1975.
- Western Blotting was first used by Towbin in 1979.
- Actually the technique is known as immunoblotting.

# What is the principle

- The method is characterized by transferring the protein, which was run on a gel by electrophoresis, onto a nitrosellulose membrane.
- This approach makes the protein stable on the membrane so that several methods including methods to detect and quantify the protein content can be employed.


(A)
sample loaded onto gel
by pipette
cathode
⊖
plastic casing
buffer
gel
⊕ anode
buffer

# First step

- Seperation step.
- The proteins in the extract are seperated by their size (molecular weight) on a gel using electrophoresis.
- SDS-PAGE Gel: Sodium dodesyl sulphate-Polyacrylamide gel electrophoresis.

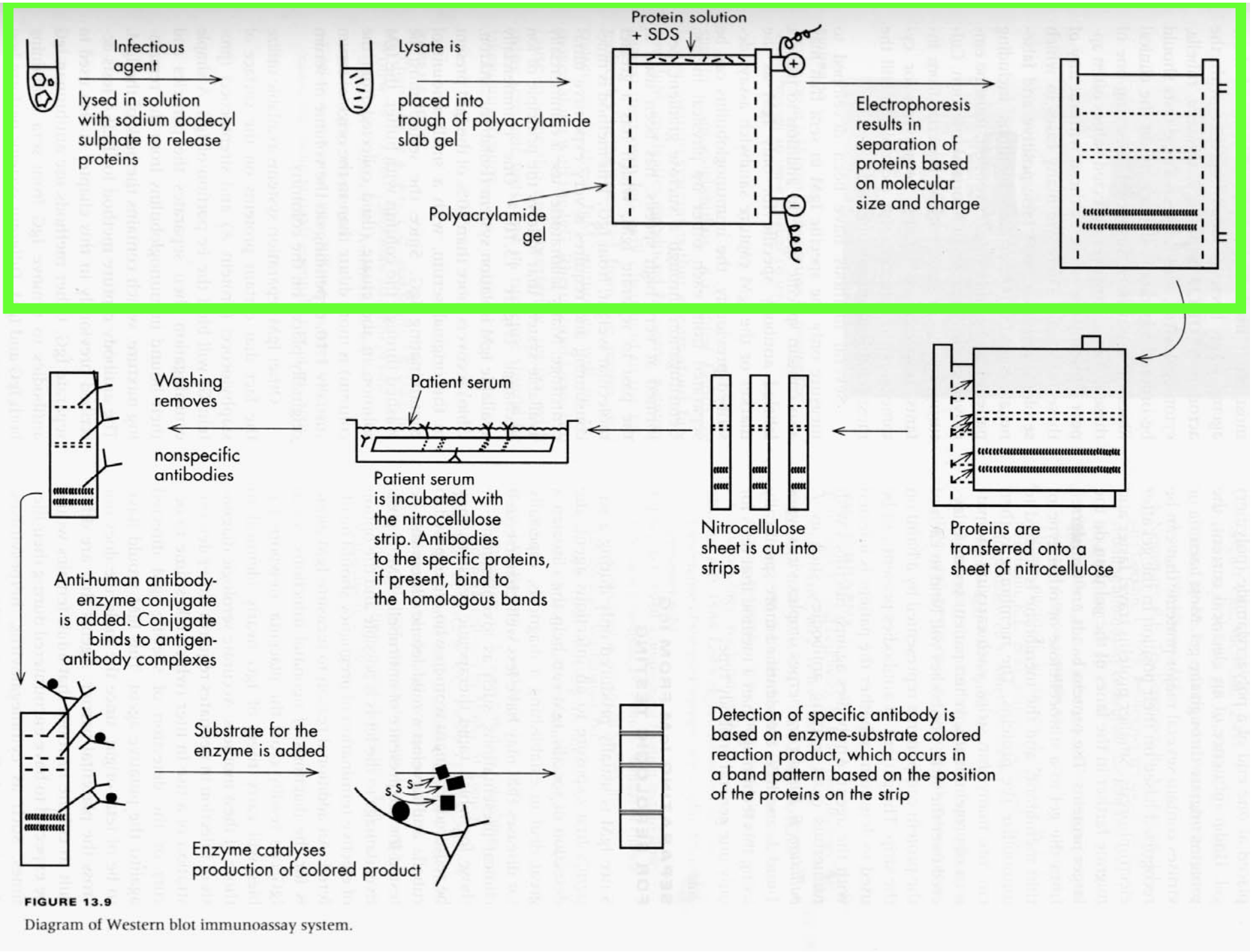



**FIGURE 13.9**

Diagram of Western blot immunoassay system.

# Second step

- Transfer step.
- The transfer of the proteins onto the nitrosellulose membrane.
- The proteins seperated on the SDS-PAGE gel are trasferred to the membrane by using electrophoresis. The localization of the proteins do not change.

# Third step

- Primary antibody incubation step.
- The primary antibodies which specifically recognize the proteins of intrest are used.

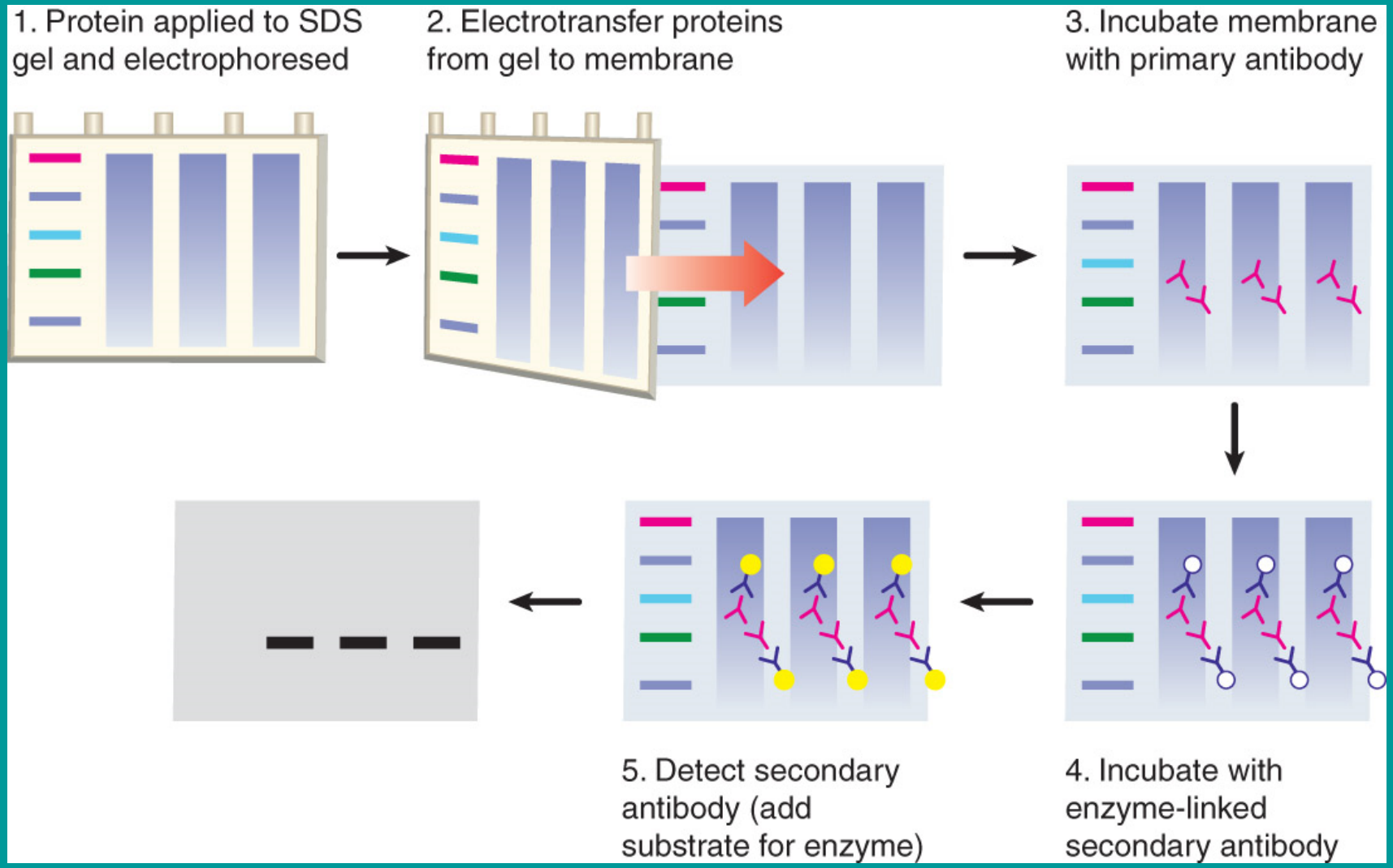
# Fourth step

- Secondary antibody incubation step.
- Use of secondary antibody which recognizes the primary antibody used in the third step.

# Fifth step

- Visualization step
- Making the antigen-antibody complex visible (staining).
  - Autoradiography (radioactive P).
  - Avidin-biotin coplex and  ve chromogen.
  - Fluoresence method.

FIGURE 23: Western blot



# Where WB is used?

- Cancer biology and pathology
- Microbiology
- Immunology
- Protein biochemistry
- Tissue studies
- Testing antibodies