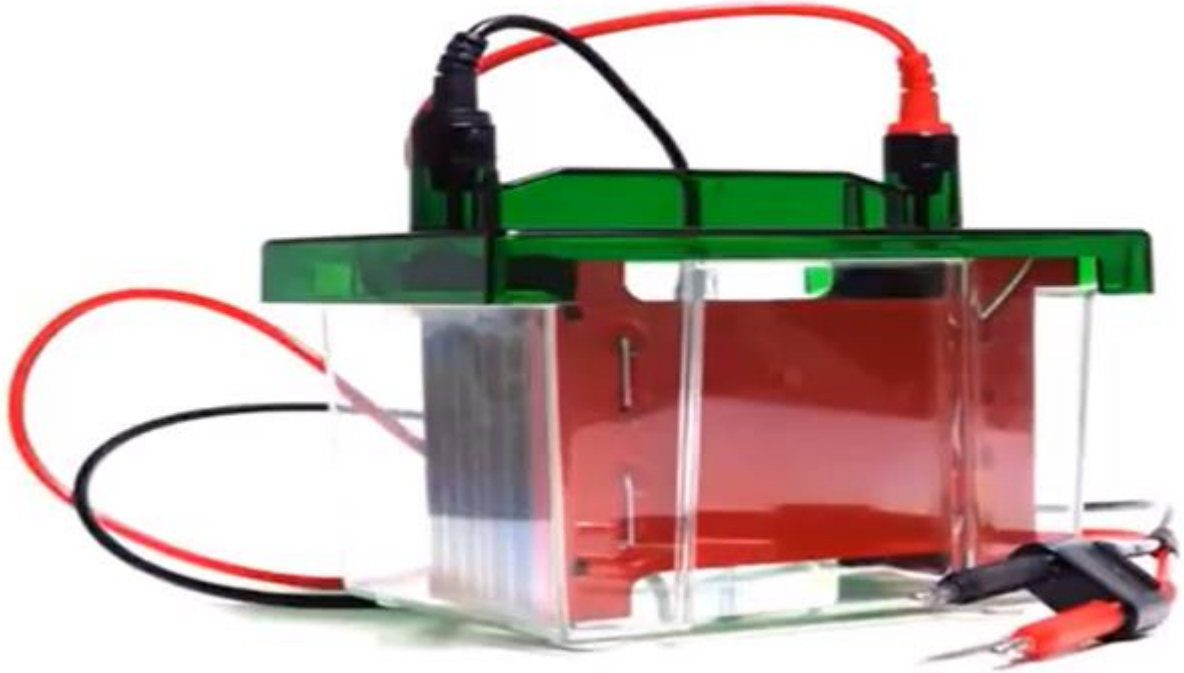
western blot



Blotting

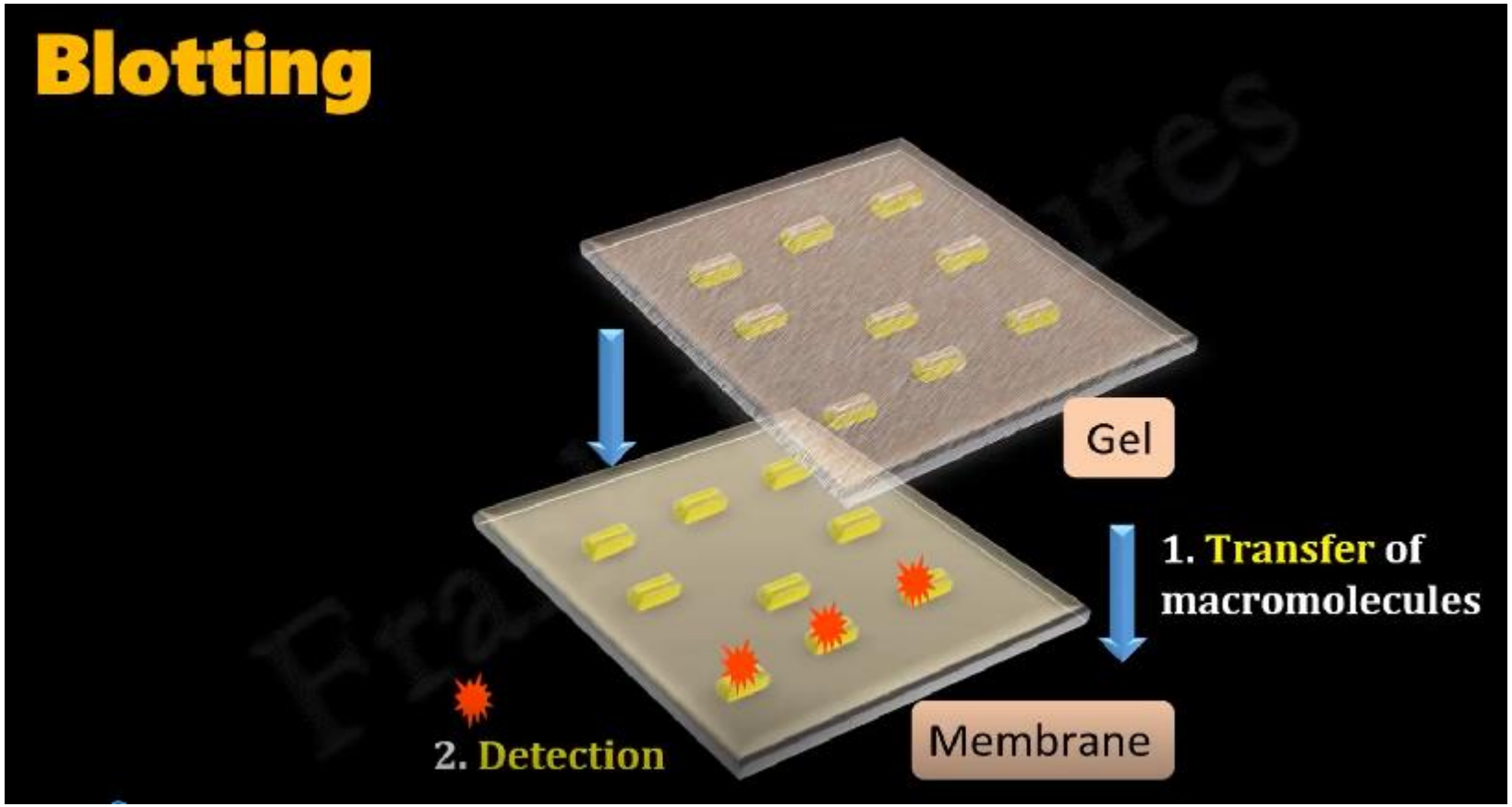
DNA : Southern Blotting

RNA : Northern Blotting

Proteins : Western Blotting



Sir Edwin Mellor Southern

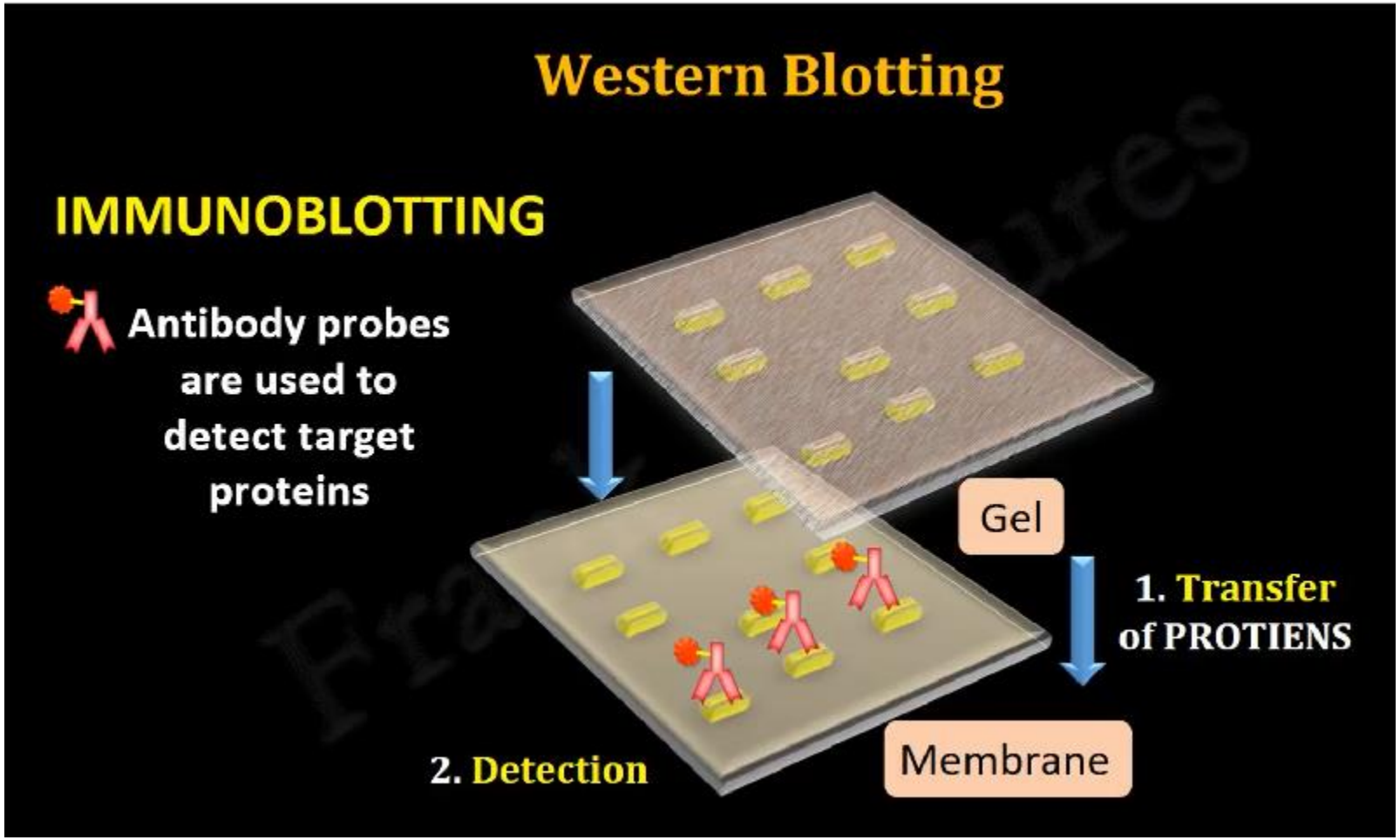

Blotting
Gel
1. Transfer of macromolecules
Membrane
2. Detection

What is a western blot?

- Blotting proteins onto a nitrocellulose membrane
- Apply primary (monoclonal mouse) antibodies
- Apply secondary (rabbit anti-mouse) antibodies – these have Horse Radish Peroxidase (HRP) attached which will catalyse the reaction of luminol→3-aminophthalate
- Enhance the fluorescence

What is it used for?

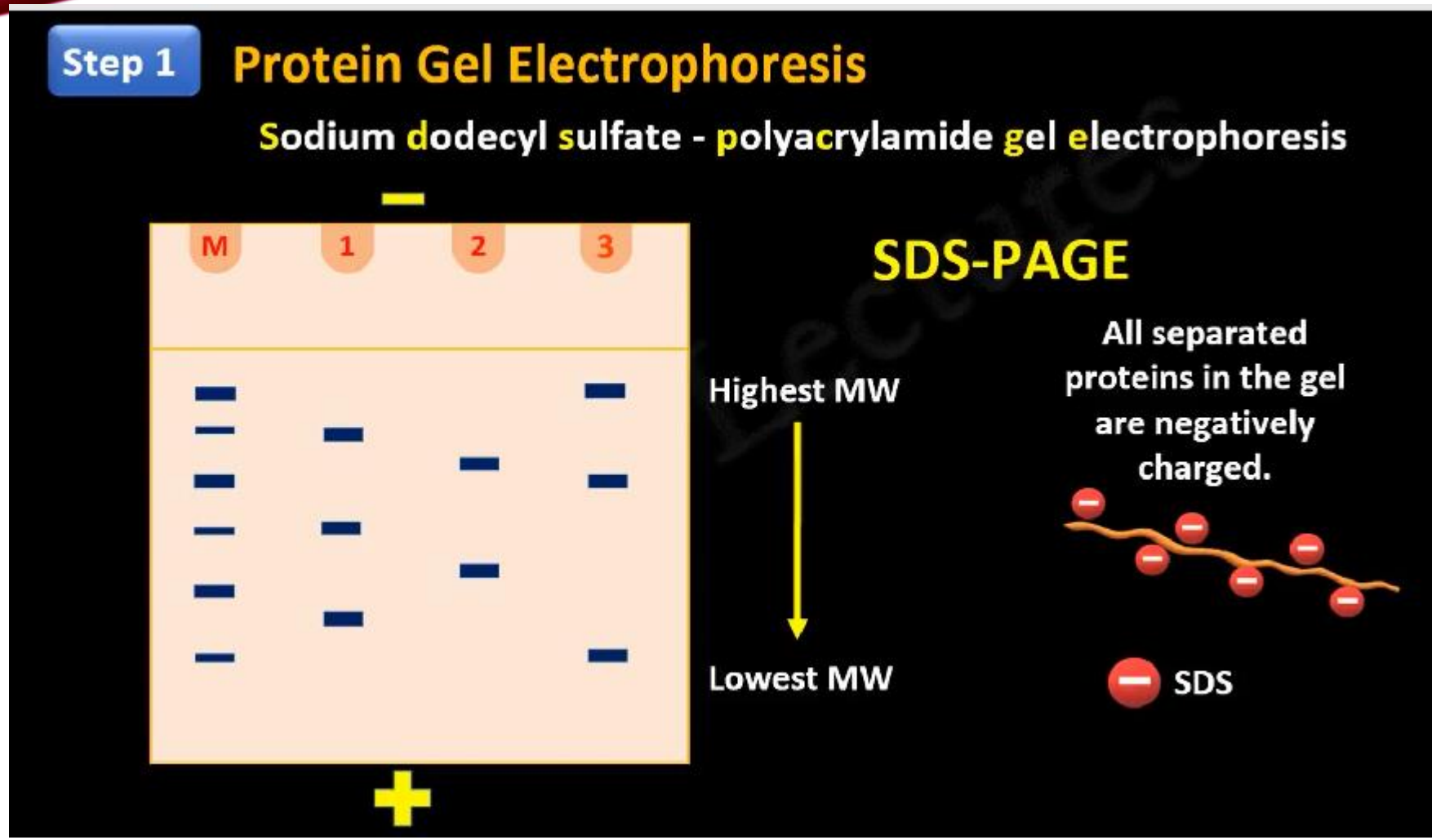
- Detection/identification of specific proteins in a sample
  - Over expression/under expression
- Used in cancer research to identify specific proteins which cause/prevent/influence tumour growth
  - Alzheimer's
  - Diagnosis for Lyme disease (ELISA)


Western Blotting
IMMUNOBLOTTING
Antibody probes are used to detect target proteins
Gel
1. Transfer of PROTIENS
Membrane
2. Detection

Western Blotting

Steps involved.....

1. Protein Gel Electrophoresis
2. Protein Transfer
3. Blocking
4. Antibody Probing
5. Detection


Step 1
Protein Gel Electrophoresis
Sodium dodecyl sulfate - polyacrylamide gel electrophoresis
M
1
2
3
SDS-PAGE
Highest MW
Lowest MW
All separated proteins in the gel are negatively charged.
SDS

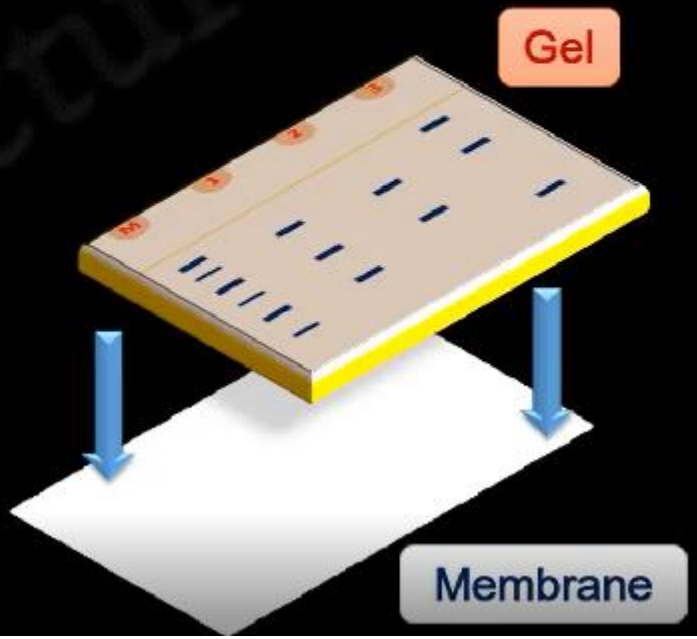
Step 2 **Protein Transfer**

Membranes

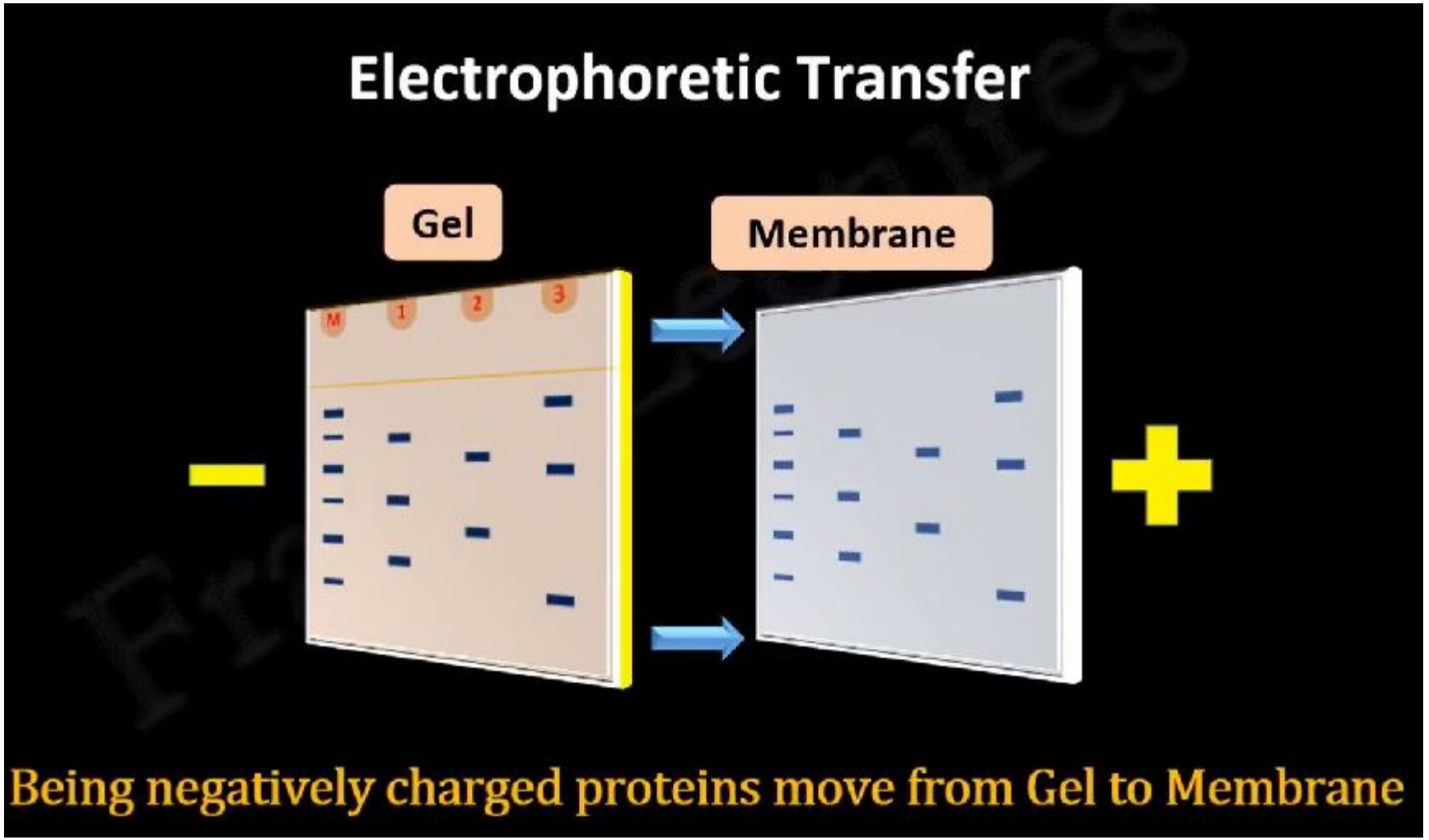
- Have **High Affinity for proteins**

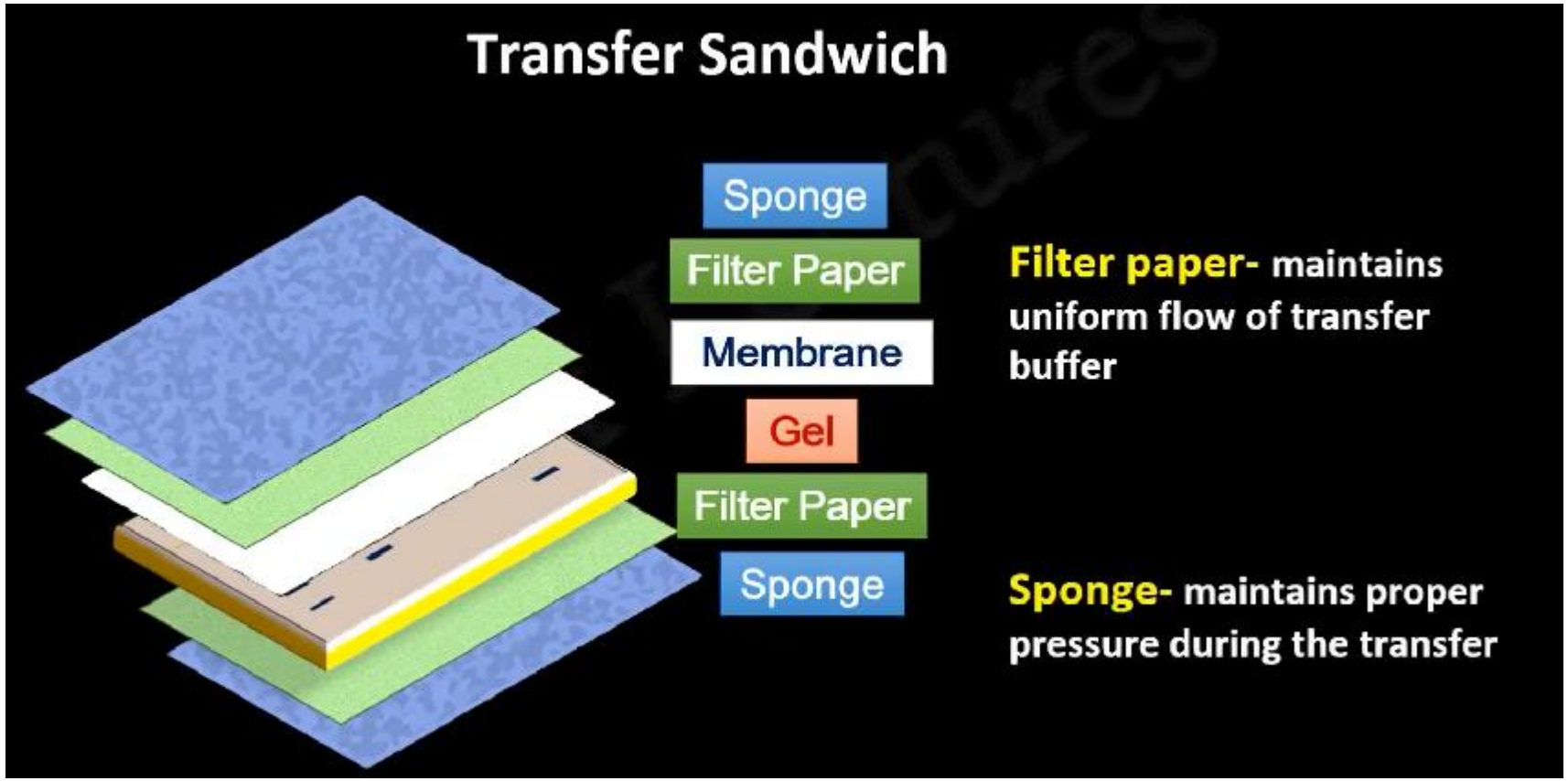
Nitrocellulose

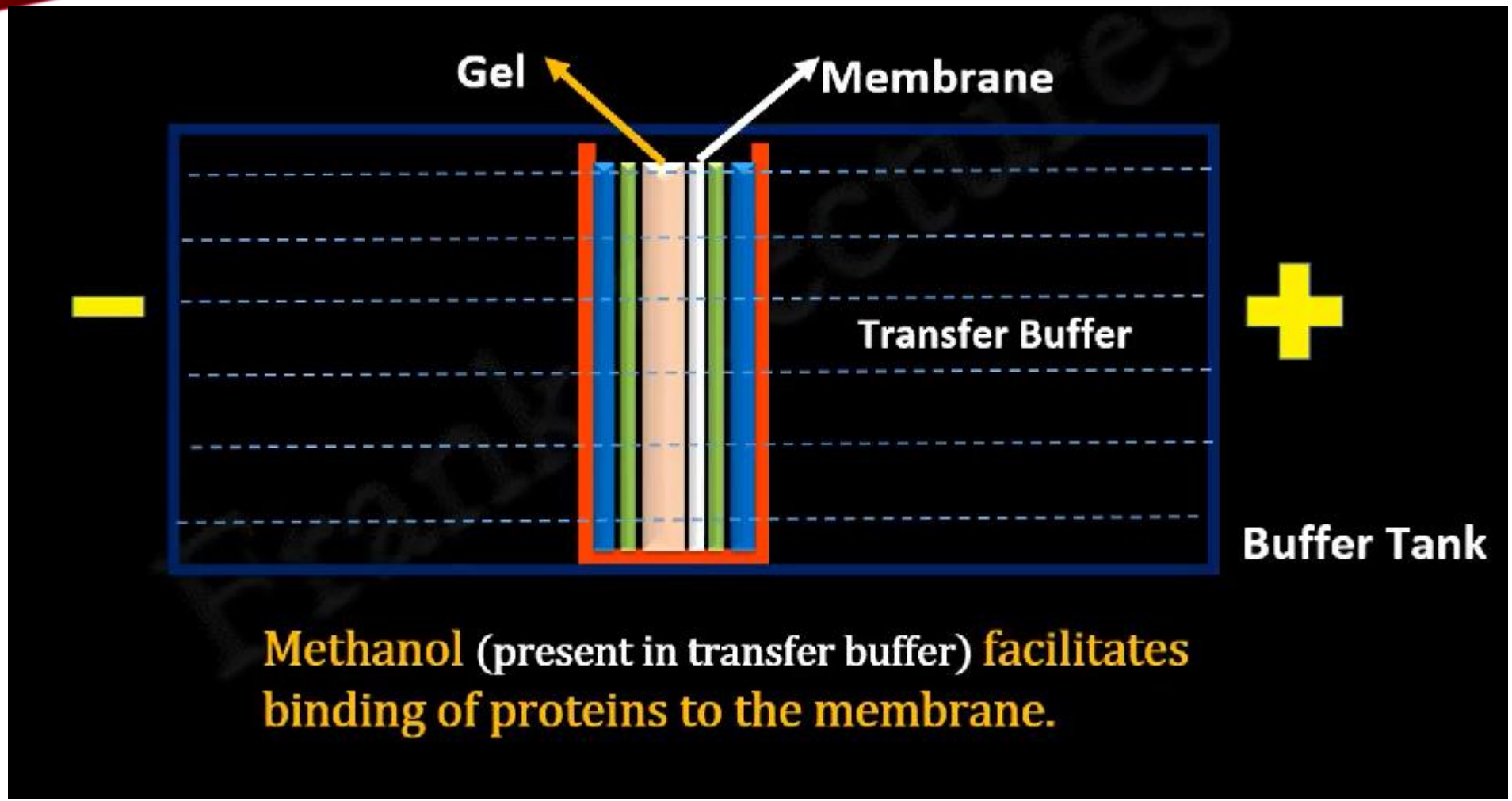
Poly**v**inylidene **di**fluoride
(PVDF)

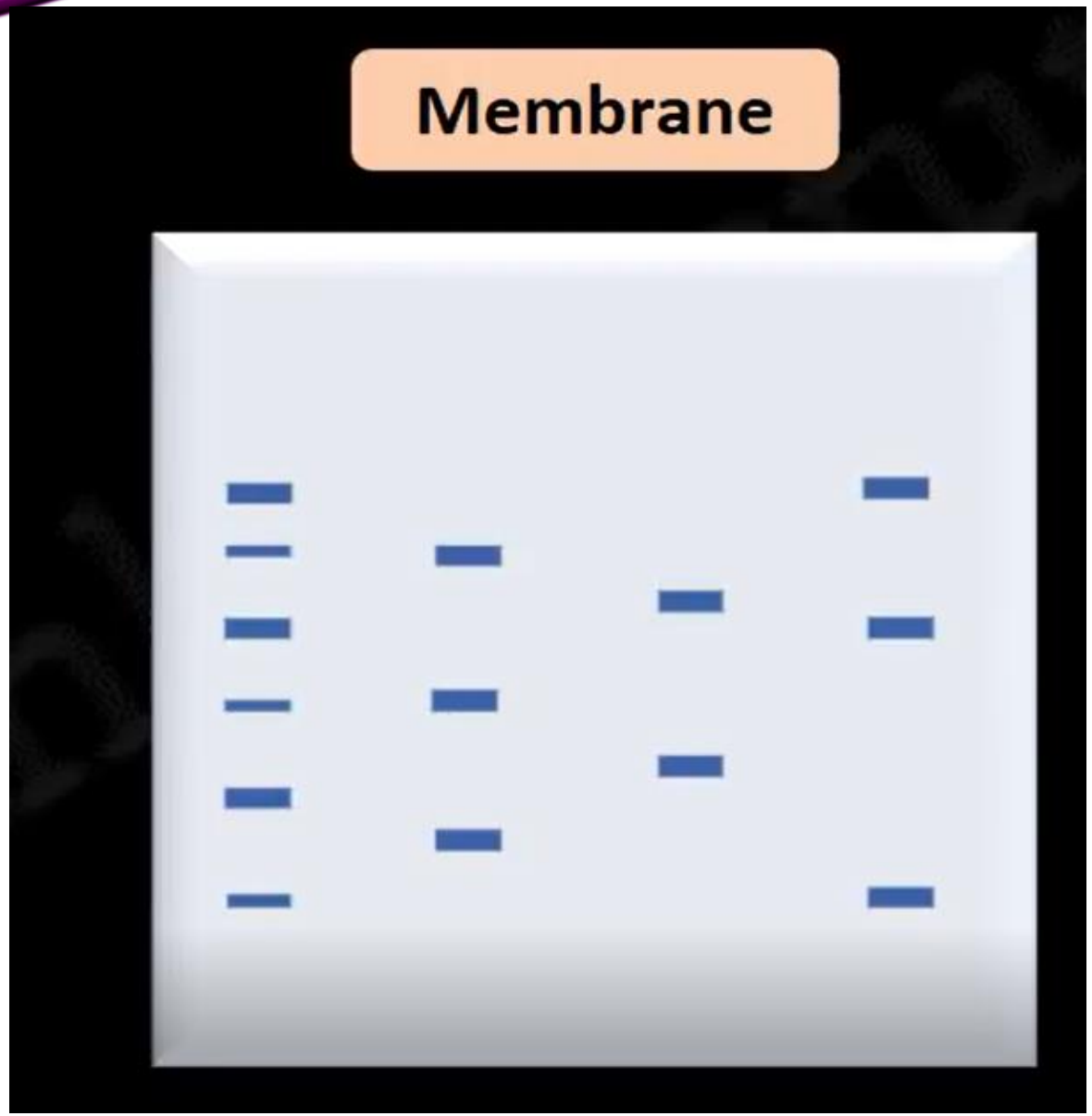


Hina Lectures


Electrophoretic Transfer
Gel
Membrane
M
1
2
3
Being negatively charged proteins move from Gel to Membrane


Transfer Sandwich
Sponge
Filter Paper
Membrane
Gel
Filter Paper
Sponge
Filter paper- maintains uniform flow of transfer buffer
Sponge- maintains proper pressure during the transfer

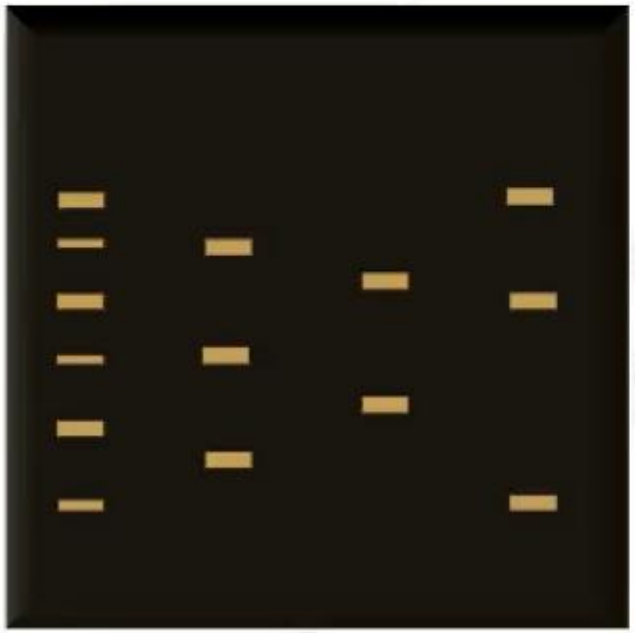

Gel
Membrane
Transfer Buffer
Buffer Tank
Methanol (present in transfer buffer) facilitates binding of proteins to the membrane.


Membrane

Step 3 Blocking

- To prevent non-specific binding of antibodies

Membrane

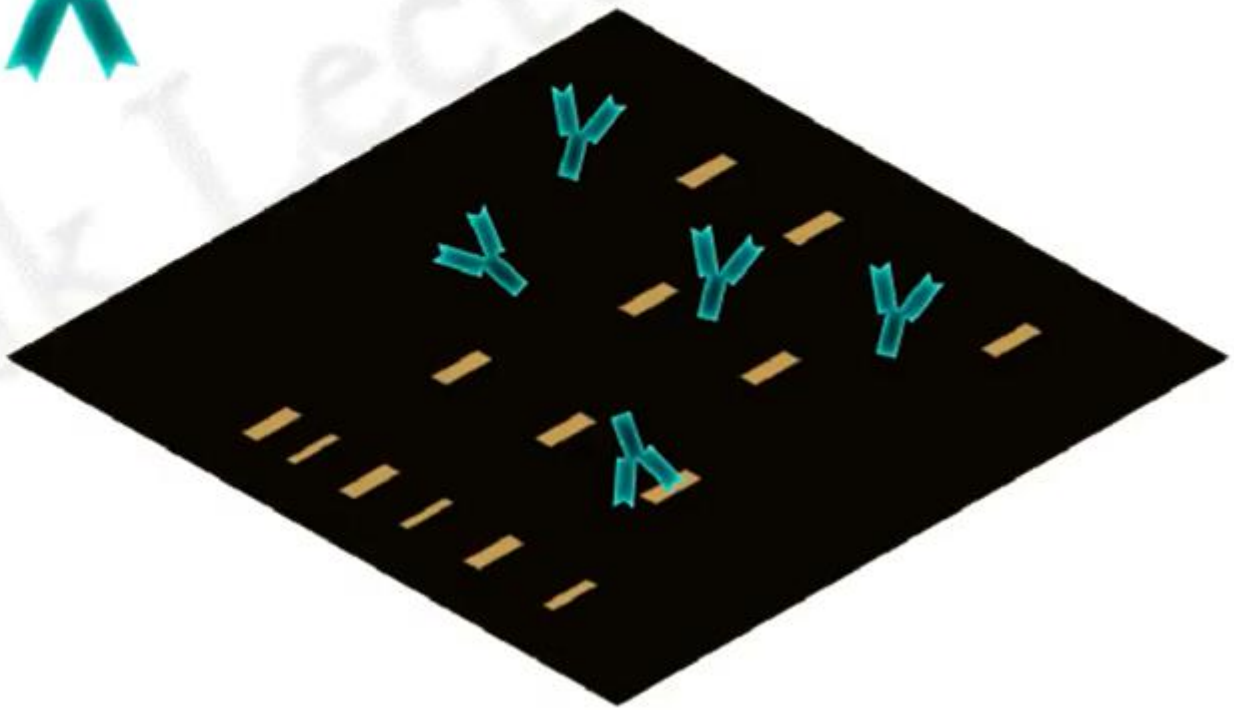


Blocking agents: non-fat milk, BSA

Step 4 Antibody Probing

Membrane is incubated with primary antibody



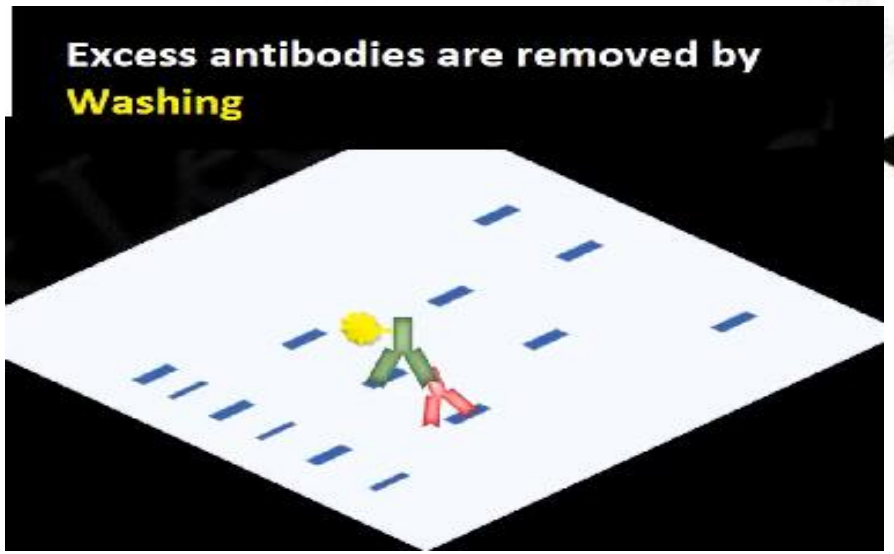


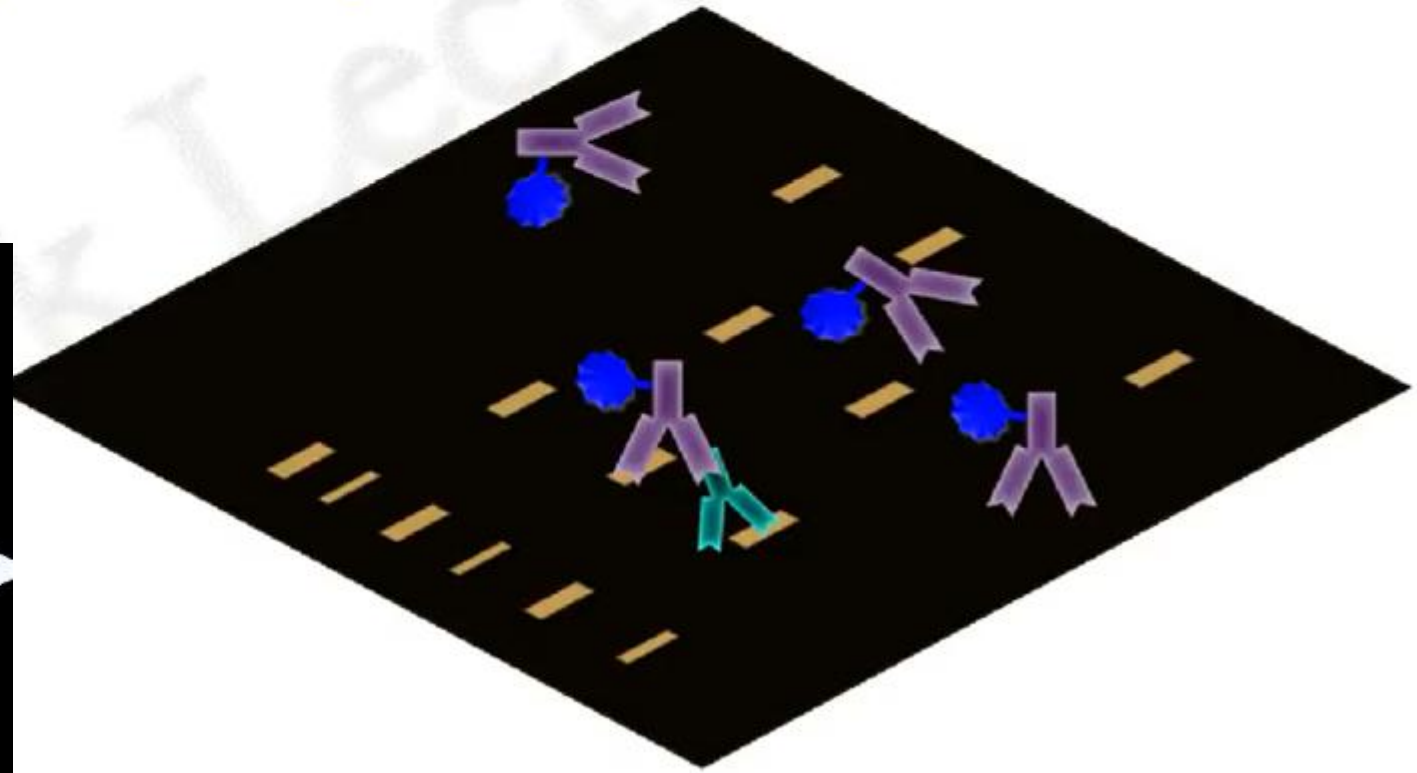
Step 5 Detection

Membrane is incubated with

LABELED secondary antibody







Step 5 **Detection**

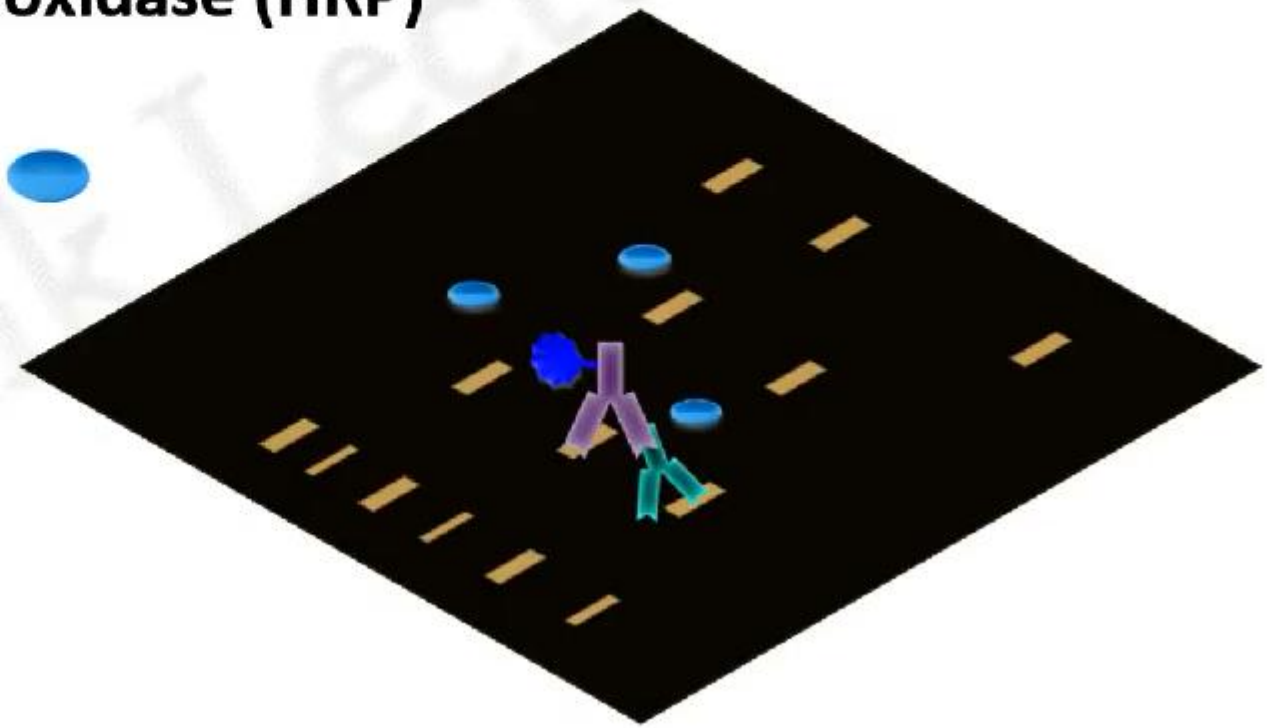


Enzyme

Horseradish Peroxidase (HRP)

Addition of Substrate

4-cholor-1-naphtol
(4CN)



Frank Lectures

Western Blotting

