Advance Genetic Engineering

Prof.Dr.Abdul Hussein M.AlFaisal
Ph.D. in Cancer Molecular Genetics
Wales University- UK.

Hybridization or Blotting of Nucleic acids and Proteins

Methods

1.Filters blotting

A. Southern Blot – DNA Blot

B. Northern Blot – RNA Blot

C. Dot Blot and colony\plaques blot

2. In Situ Hybridization

3. Western Blot – Proteins Blot

Southern Blot – DNA Blot

Procedure

1. DNA extraction
2. DNA digestion with restriction enzymes
3. Agarose Gel electrophoresis run for the digested DNA
4. Staining and Treatment of gel

A. Treatment with 0.25 M of Hcl for 10 mins

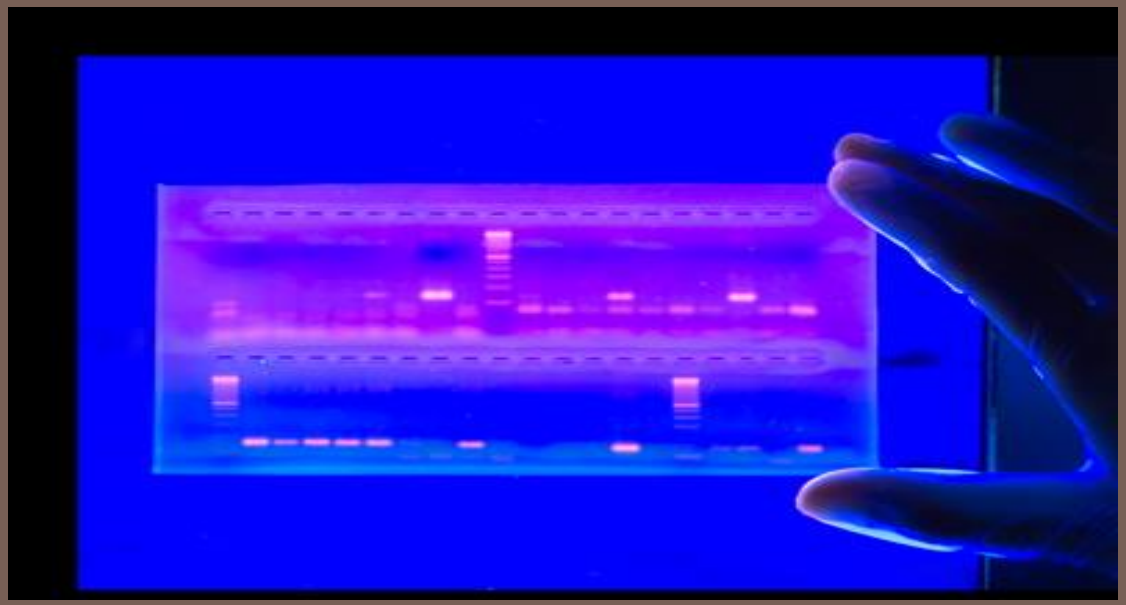
B. Treatment with 0.5 M NaoH+1.5 M Nacl solution for 60 mins

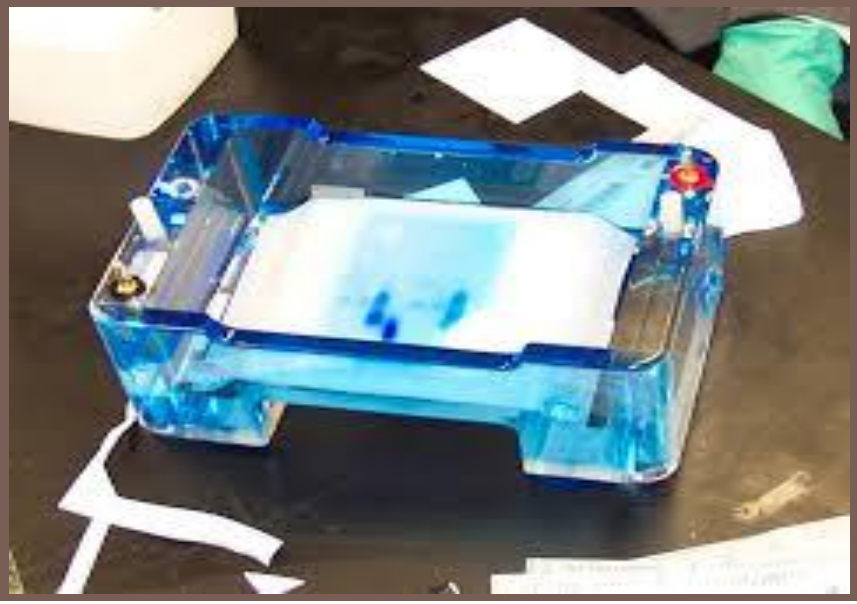
C. Treatment with neutralizing solution – 1.5 M Nacl+0.5 M Tris-cl+0.001 M EDTA,PH7.2

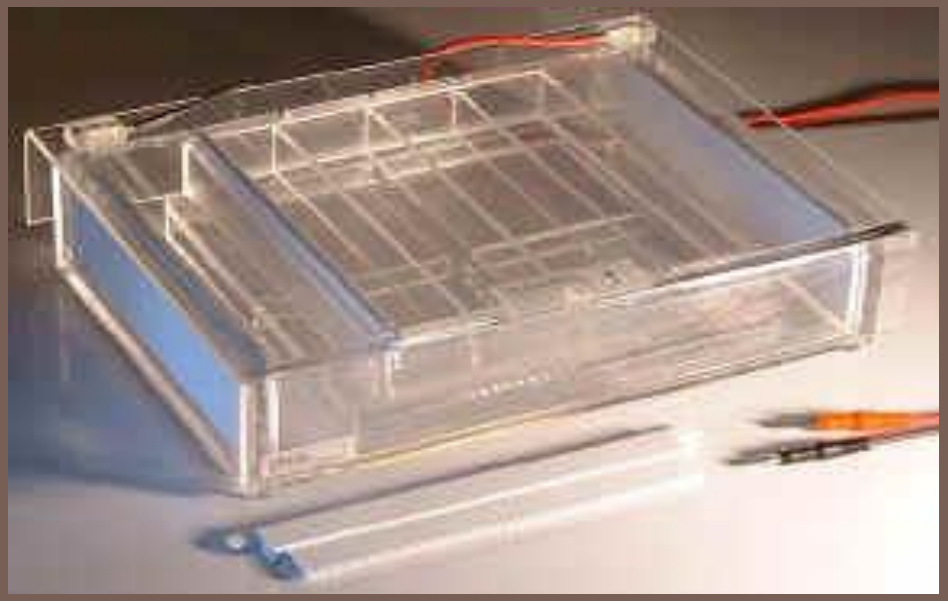
for 45 mins…….gel is fragile

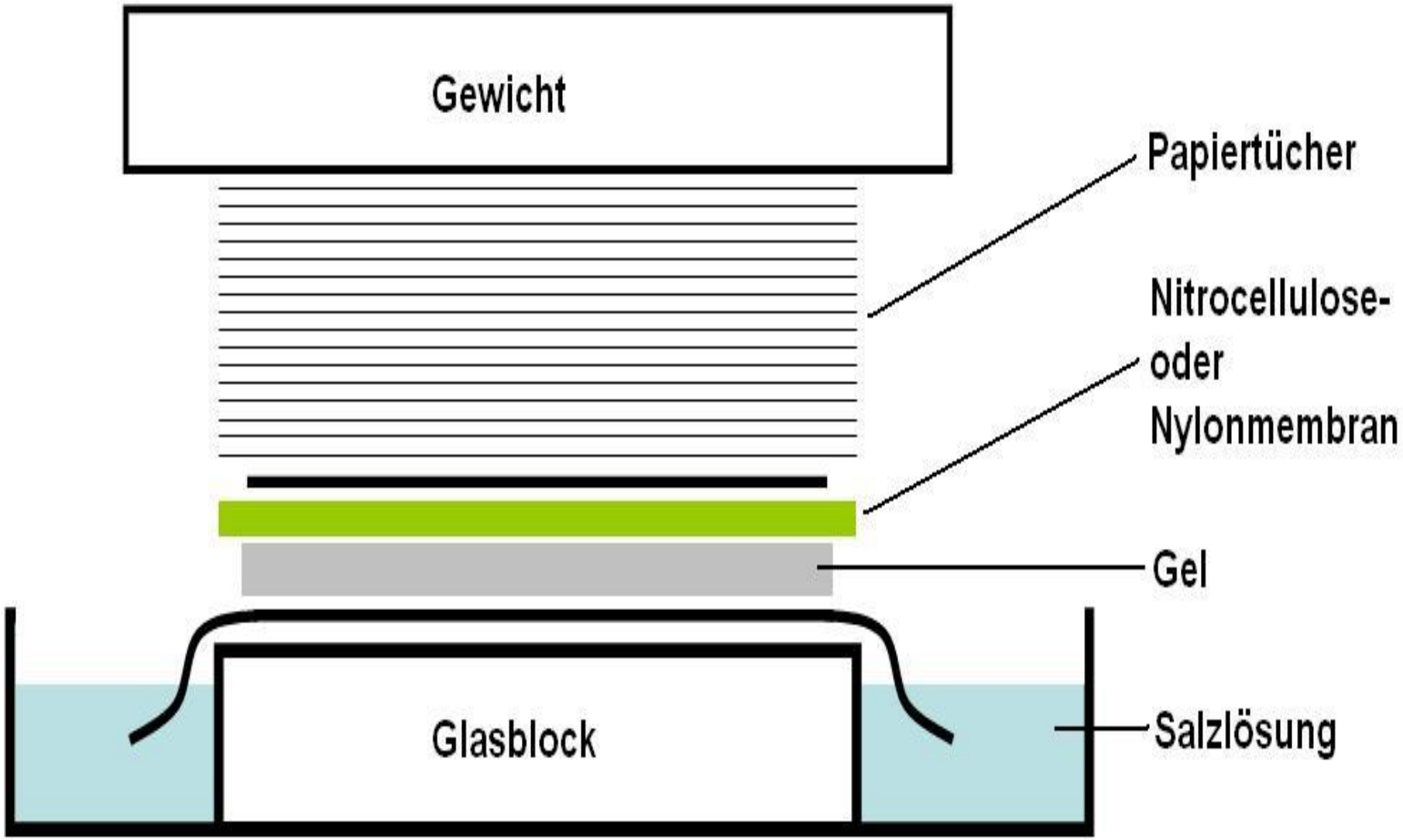
5. Cover the surface of the stage of the electrophoresis tank with filter paper, remove the air bubbles and keep the ends of the filter immerse in SSC buffer
6. Transfer gel gently to electrophoresis tank
7. Add electrophoresis buffer -10X SSC-
8. Immerse a gel size nitrocellulose or hybond filter in the SSC buffer for 1 min
9. Add the gel size nitrocellulose or hybond filter over the agarose gel and remove the air bubbles
10. Add 1-2 filter papers over nitro filter then add a bunch of tissue papers and a heavy glass
11. Keep the system working for ovenight

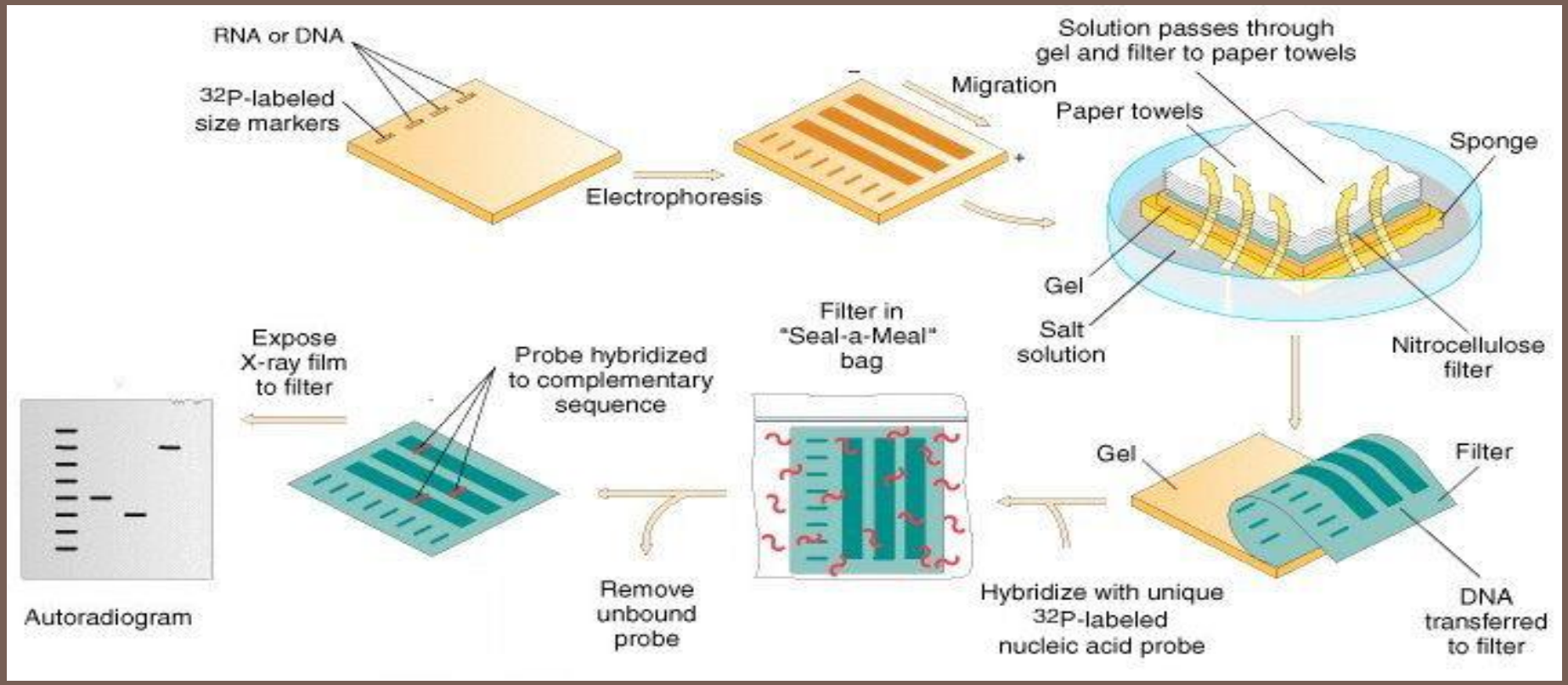
11. Remove the glass, tissue and filter papers
12. Remove the nitro filter and dry in a hot plate -80 C- with vacuum to fix DNA fragments
13. Go for hybridization





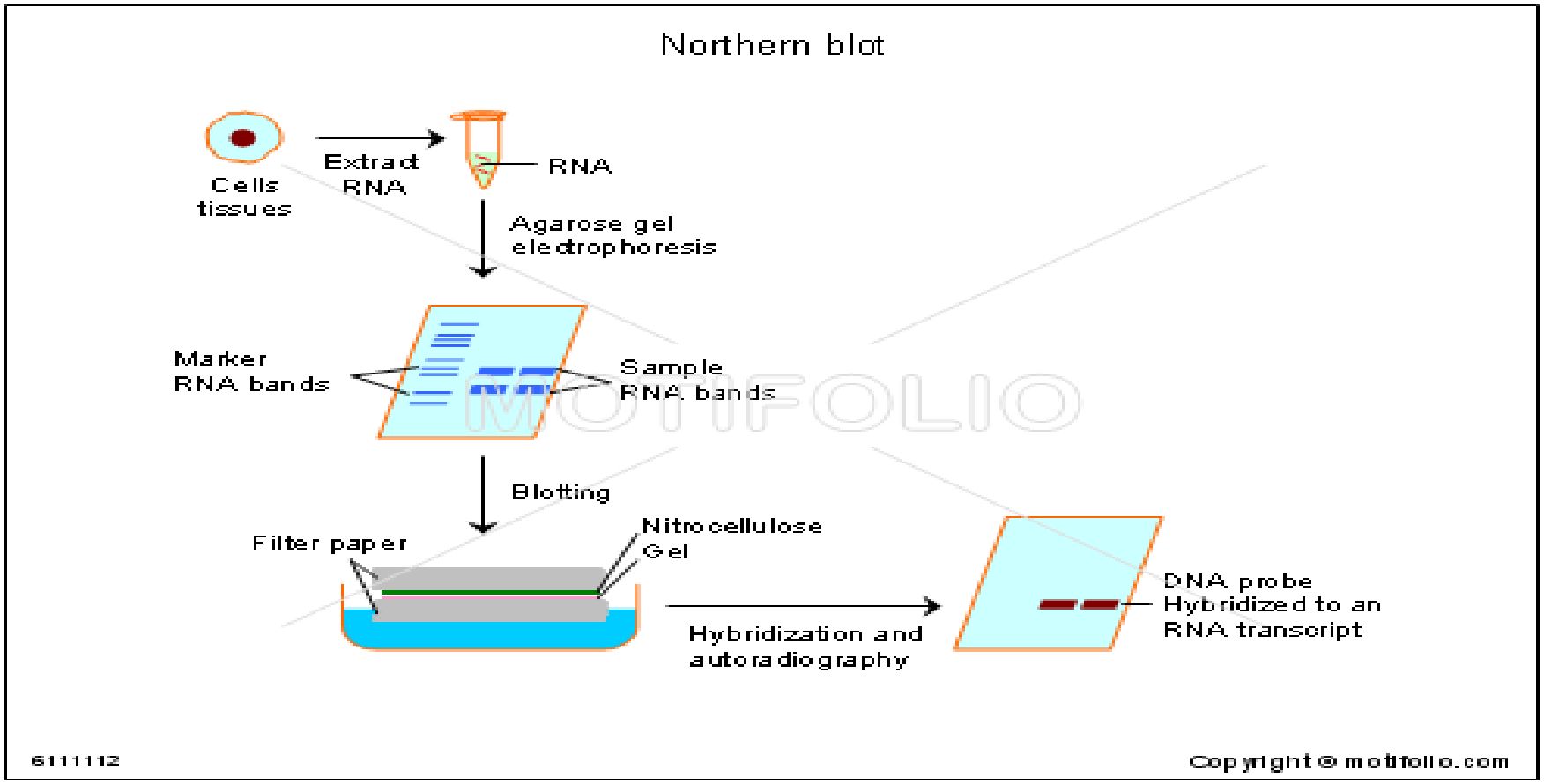



Gewicht
Papiertücher
Nitrocellulose-
oder
Nylonmembran
Gel
Glasblock
Salzlösung


RNA or DNA
32P-labeled size markers
Electrophoresis
Migration
Solution passes through gel and filter to paper towels
Paper towels
Sponge
Gel
Salt solution
Nitrocellulose filter
Filter in "Seal-a-Meal" bag
Probe hybridized to complementary sequence
Expose X-ray film to filter
Autoradiogram
Remove unbound probe
Hybridize with unique 32P-labeled nucleic acid probe
Gel
Filter
DNA transferred to filter

Northern Blot – RNA Blot

RNA filter blotting



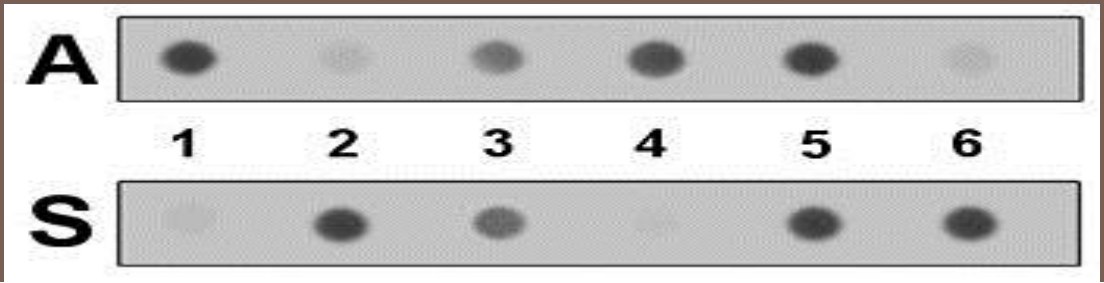
Differences between:

Southern and Northern blotting ????

Dot Blot and colony\plaques blot

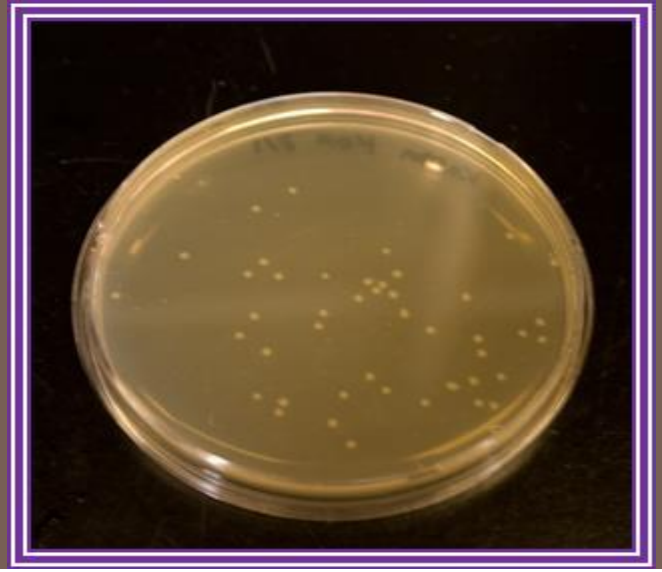
Dot Blot

1. Cut a strip of nitro or hybond paper
2. Add DNA as blots –drops- without digestion with enzyme
3. Treat paper with 0.25 M Hcl
4. Treat paper with denaturation solution for 1 hr
5. Fix DNA
6. Go to hybridization

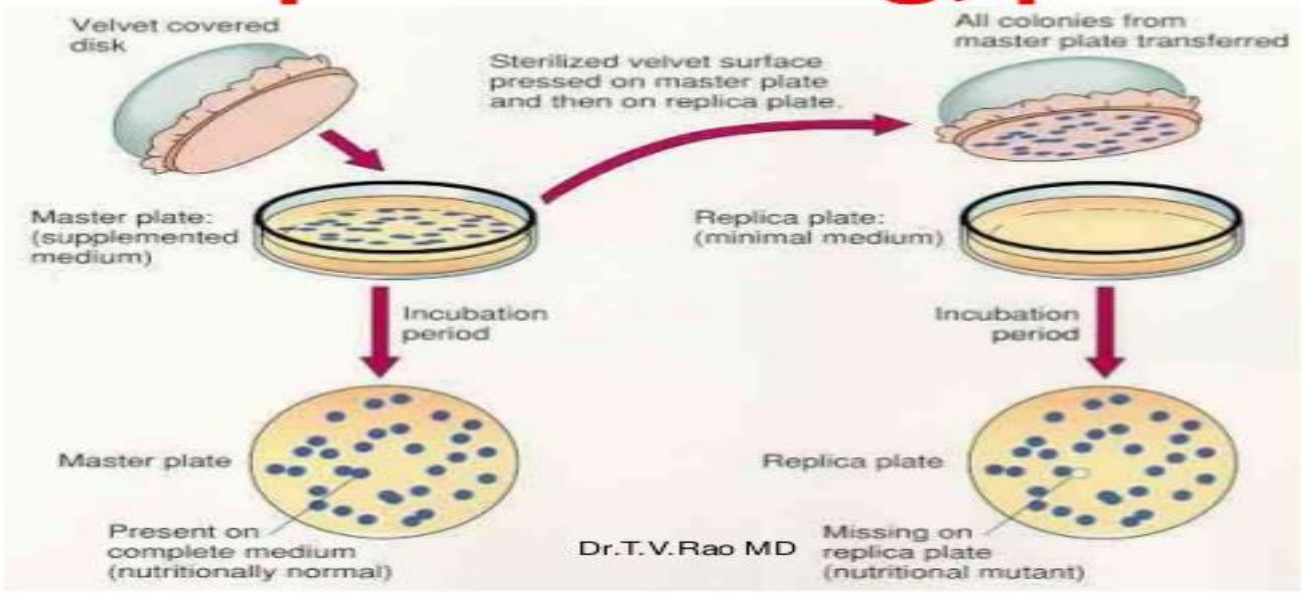


colony\plaques blot

1. Grow the bacterial clones
2. Transfer bacteria colonies or phage plaques to nitro or hybond filter
3. Transfer blotted filter to a Petri dish with filter saturated with chloroform-add blotted filter over the saturated filter and close the dish
4. After 20-30 mins remove blot filter, dry, add over saturated filter with 0.25 M Hcl for 5 mins
5. Treat blot filter with de-naturation solution for 5 mins then transfer blot filter to neutralizing solution
6. Go to hybridization

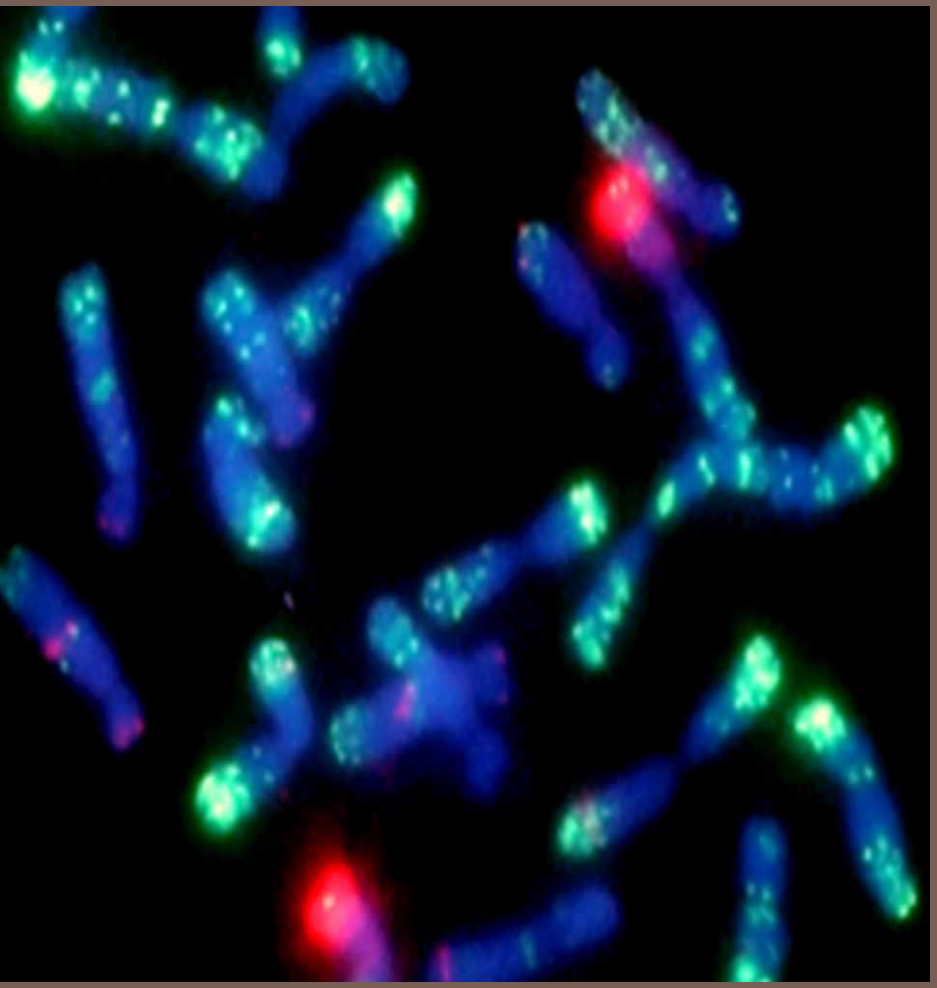


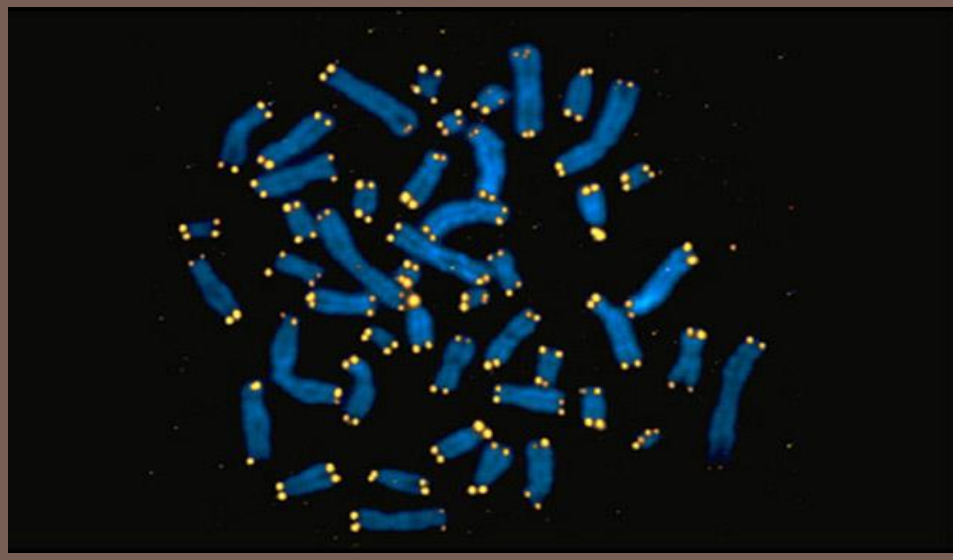
Replica Plating, pt. 2

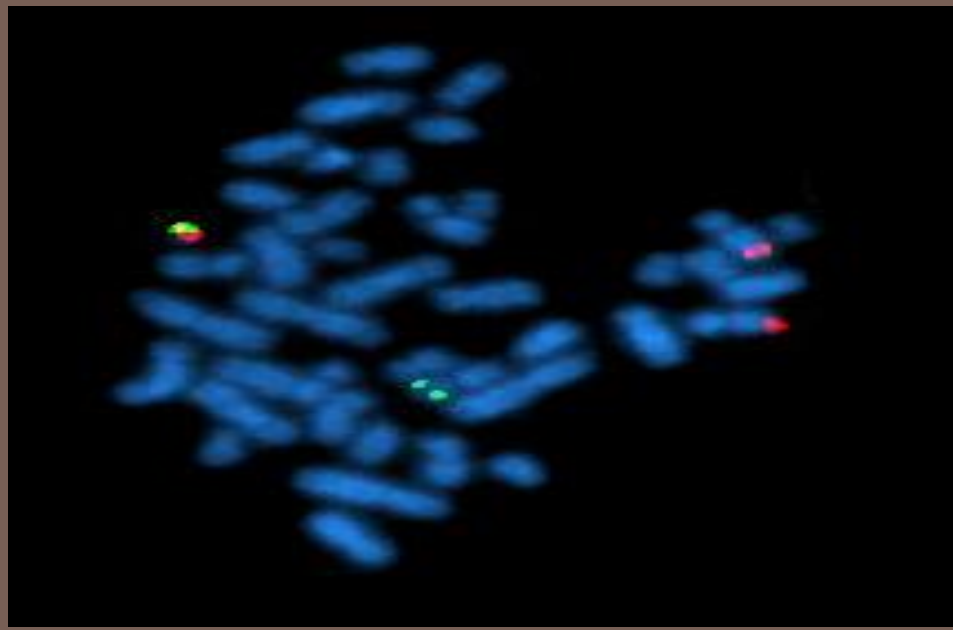


Chromosomes *In Situ* Hybridization

1. Grow divided cells until metaphases on glass slides
2. Lyse cells to free chromosomes
3. Fix chromosomes using glacial acetic acid+ethanol solution ,1:3
4. Treat with 100 ug\ml of Rnase enzyme+incubation at 37C for 1 hr
5. Wash with serial ethanol
6. Wash with 70% formamide solution or 15% NaoH for 3-5 mins
7. Wash with serial ethanol
8. Go for hybridization
9. Wash slides then Dry them and in a dark room dip slides in photographic emulsion
10. Store slides in dark box at -20C for 7 days or more
11. Treat slides photographically then stain slides with Giemsa stain
12. Check results with microscope





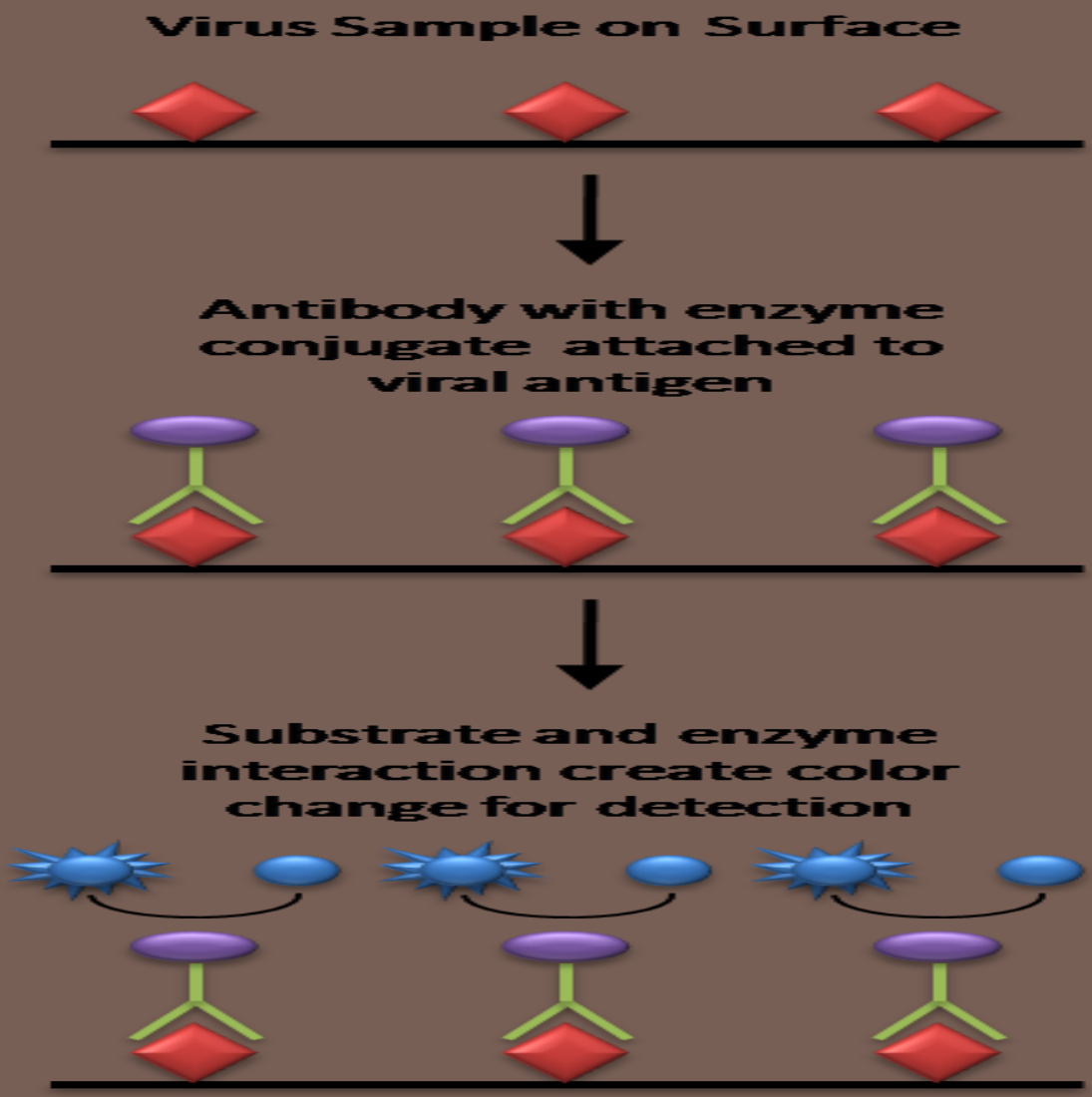


Proteins Blot

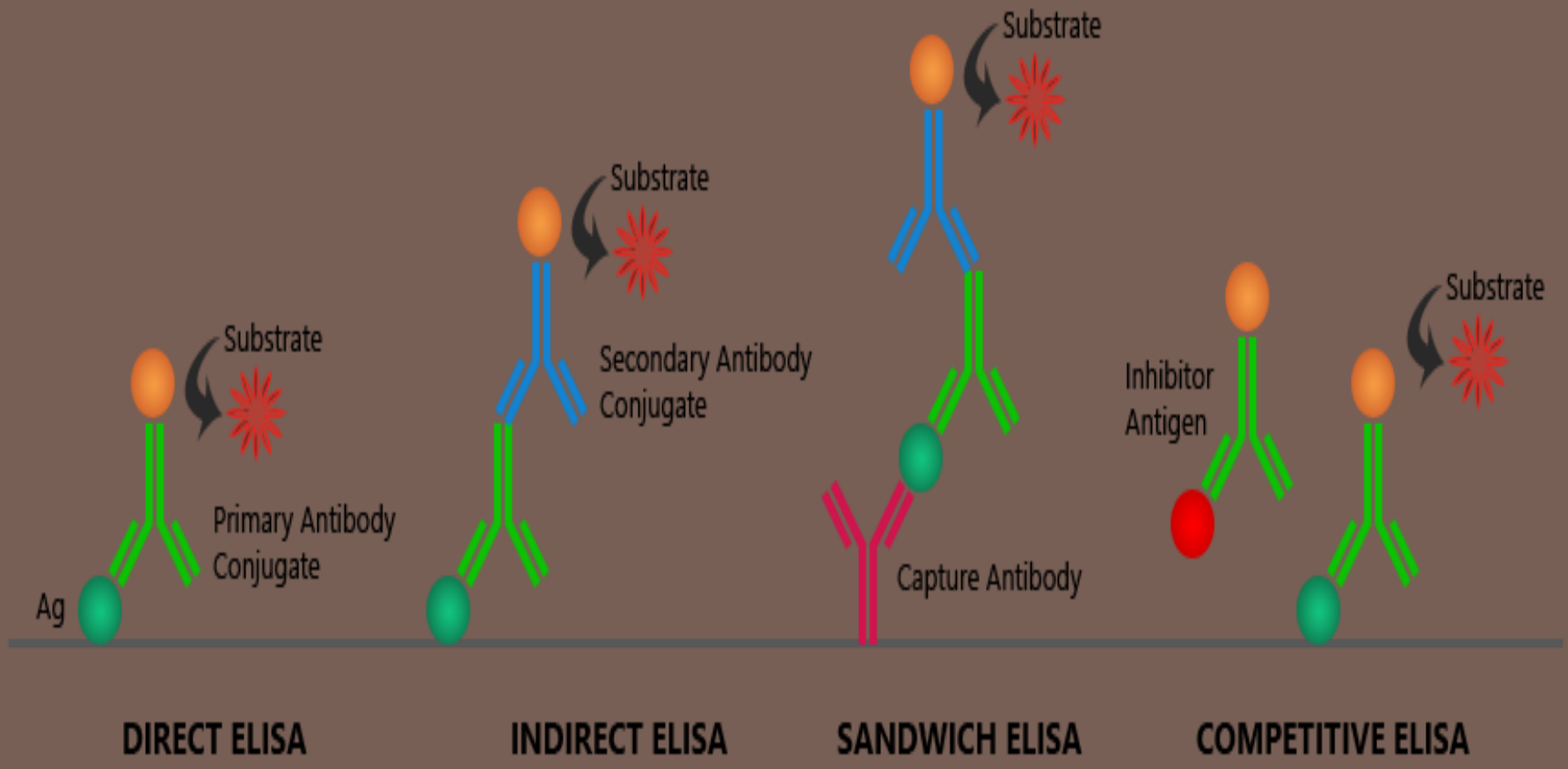
1. Solid phase immuno-assay
2. Enzyme linked immuno-sorbent assay- ELISA
3. Western blot

Solid phase immuno-assay

1. Immerse the filter in a specific protein isotope labelled antibody
2. Dry filter and mix the protein mixture over all the filter
3. Incubate at 37c for 1 hr
4. Wash filter to remove the excess materials
5. Dry filter
6. Check using Geiger system


Virus Sample on Surface
Antibody with enzyme conjugate attached to viral antigen
Substrate and enzyme interaction create color change for detection

Enzyme linked immuno-sorbent assay- ELISA



Western blot

1. Run proteins mixture via SDS polyacrilamid gel
2. Transfer proteins bands to filter as in Southern blot
3. Dry and fix samples
4. Go to hybridization

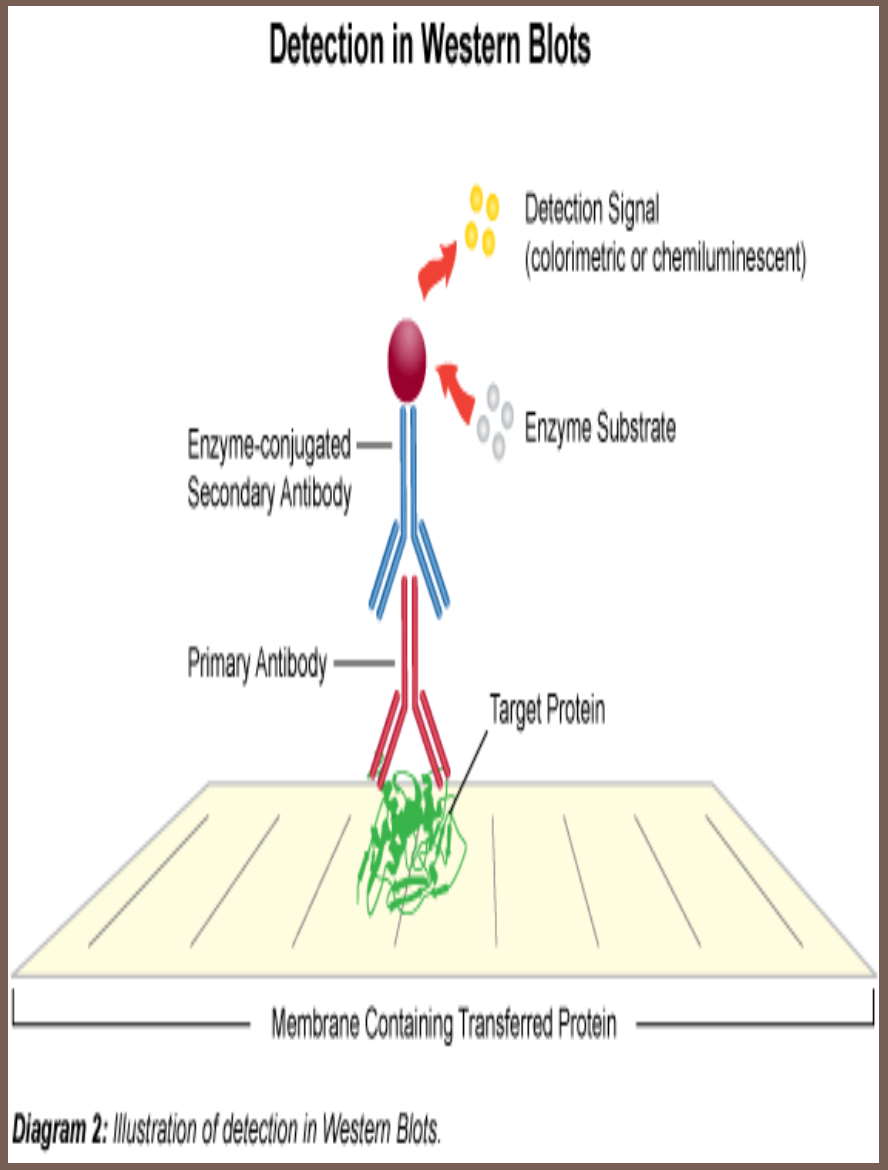
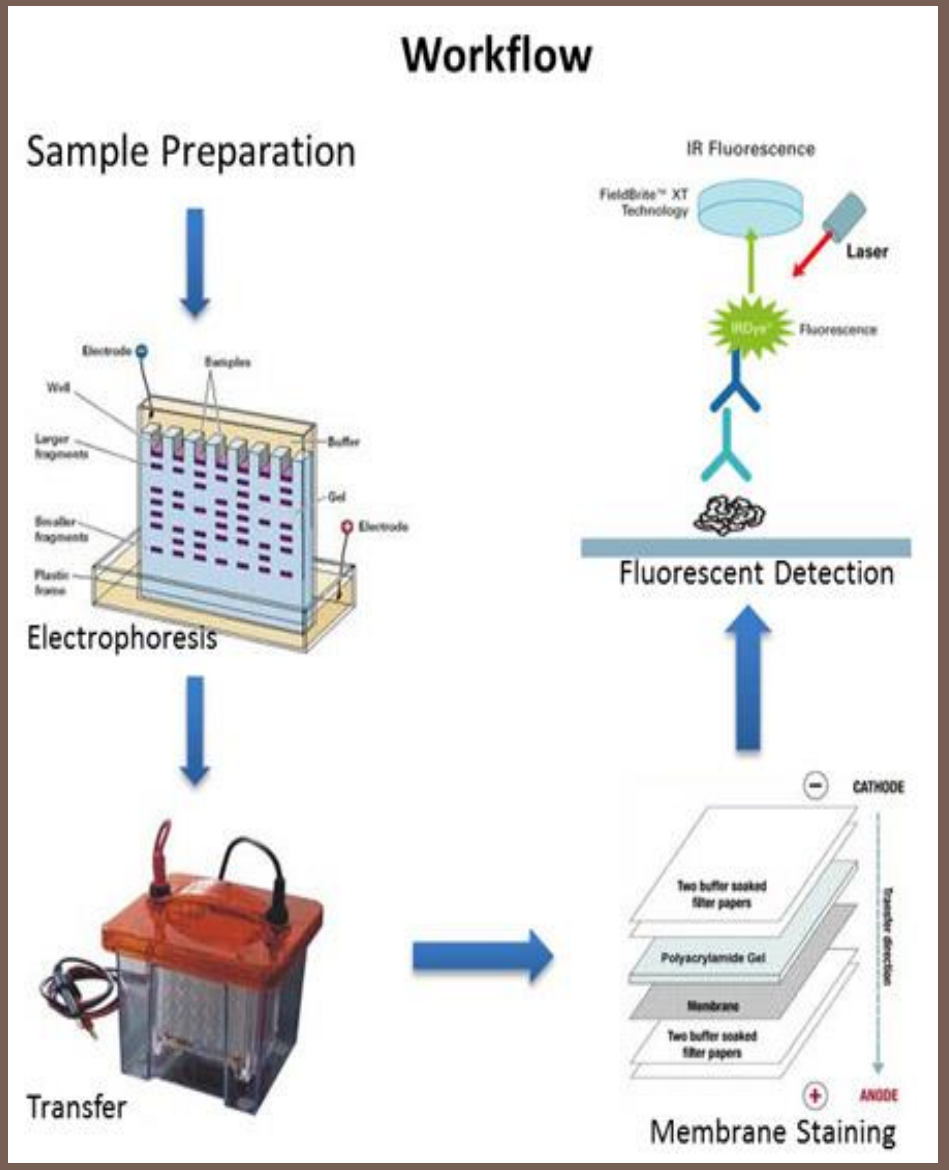


Diagram 2: *Illustration of detection in Western Blots.*




Thank you for listening