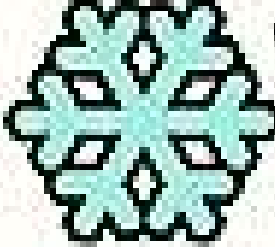



Blotting techniques

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SN  W

DR  P

S	-	SOUTHERN	-	DNA	-	D
N	-	NORTHERN	-	RNA	-	R
⊙	-	○○○○○○○○	-	○○○○	-	⊙
W	-	WESTERN	-	PROTEIN	-	P



Southern blotting

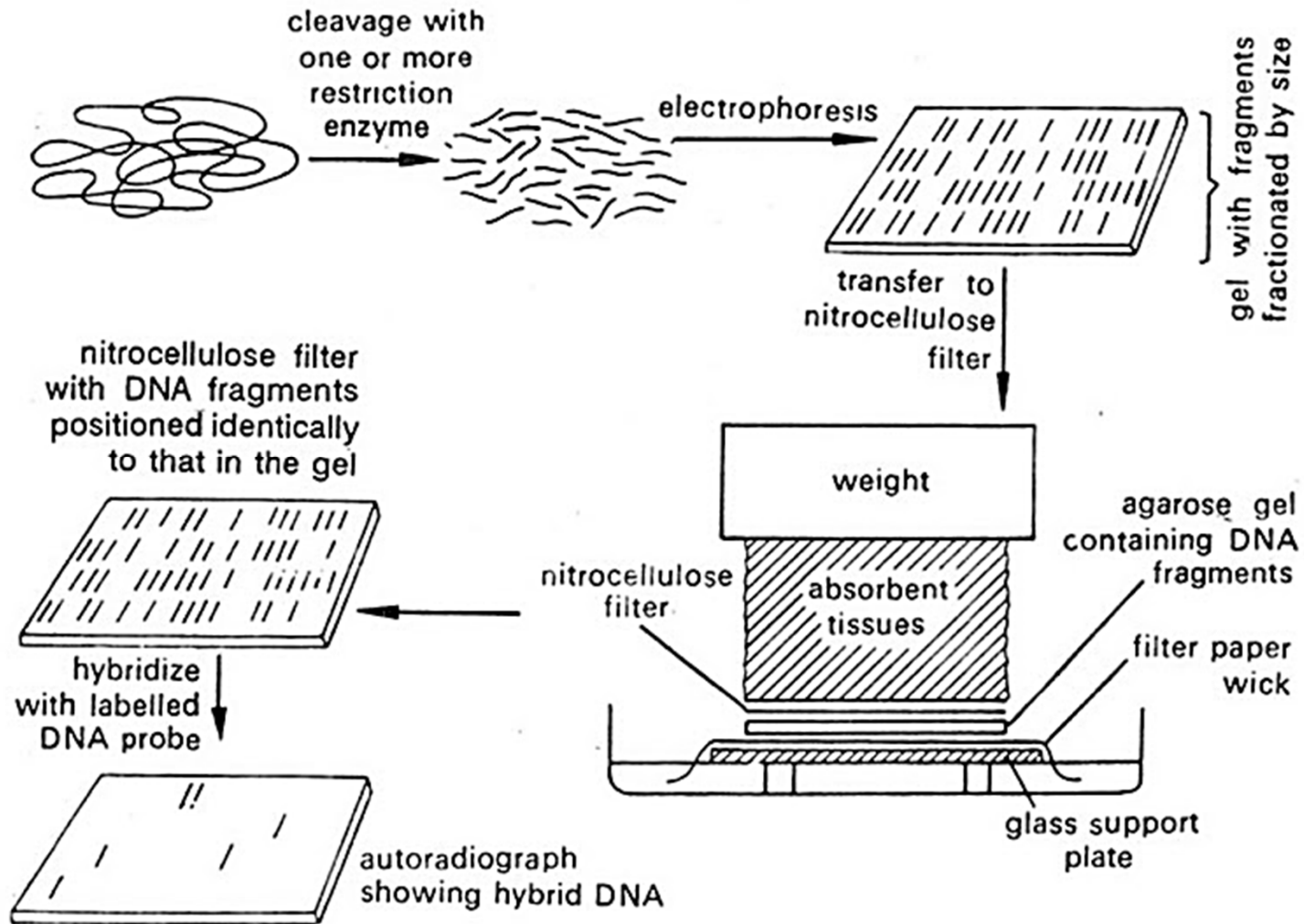


Ed Southern

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.

Procedure



Steps of Blotting

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DNA extraction

Restriction Digestion

Gel electrophoresis

Pre-treatments

Membrane transfer

Immobilization

Prehybridization

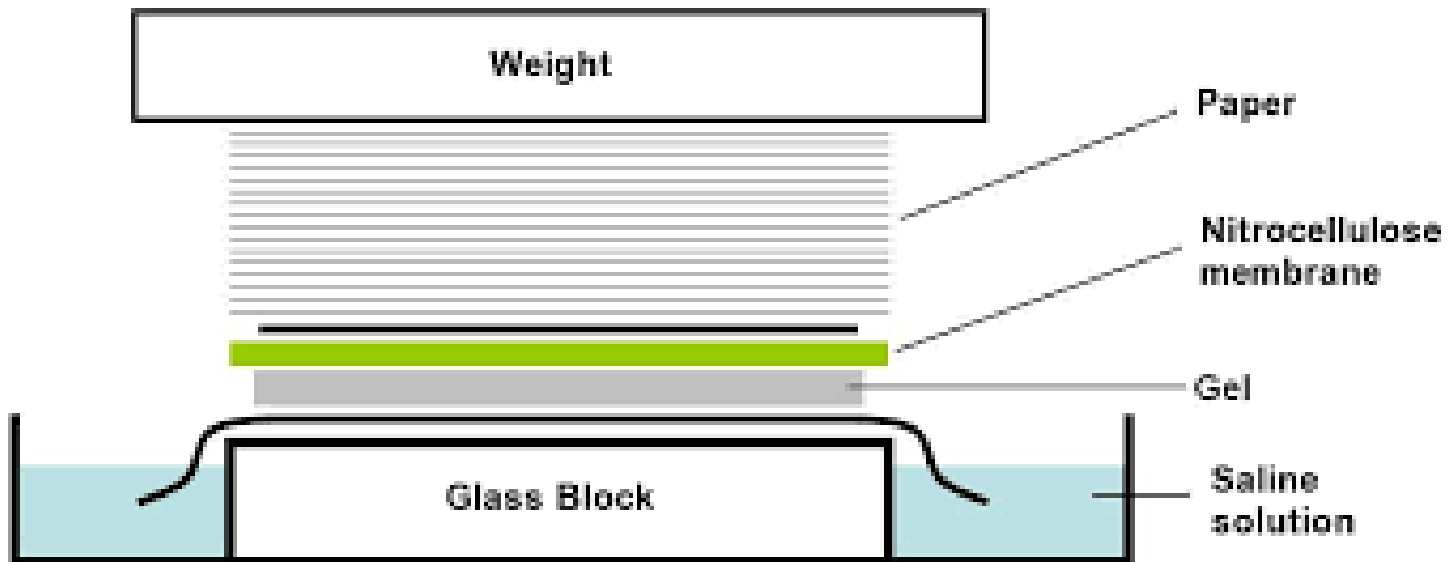
Probe the Membrane

Visualization

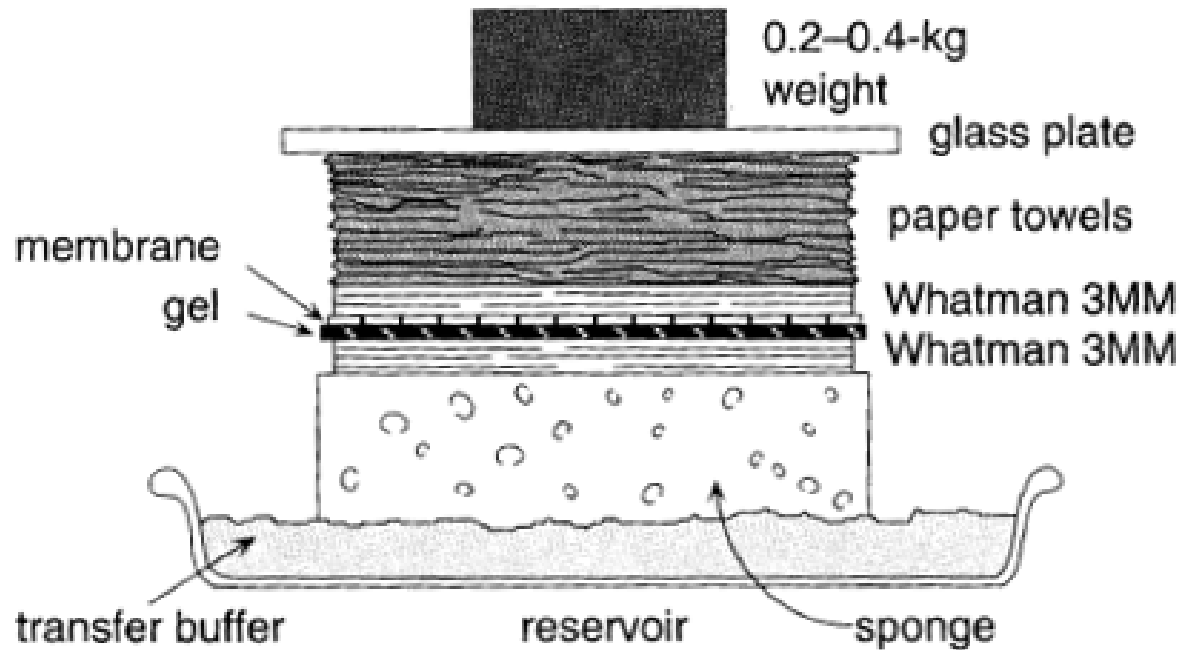
Pretreatment

- Soaking the gel in 0.25 mol L^{-1} **HCl** for 30 mins, which results in a **small amount of depurination** – cleavage of the β -N-glycosidic bond between purine bases (adenine or guanine) and the sugar component of their nucleotides.
- **Alkaline solution** that denatures the double-stranded DNA molecules by breakage of their hydrogen bonds, so the molecules become **single-stranded**.

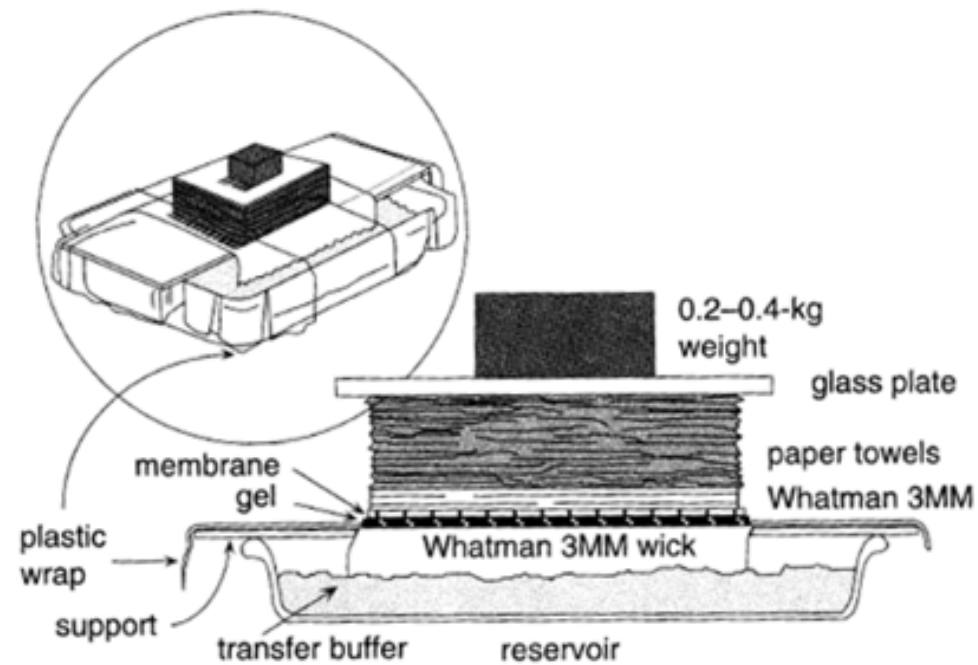
Arrangement for Southern Blot



Sponge Method of Transfer



Wick Method of transfer



Immobilization

- **Nylon membrane:** Treated with UV light. This help to cross links (via covalent bonds) the DNA to the membrane.
- **Nitrocellulose Membrane:** Can be baked at about 80°C (noncovalent but semi-permanent attachment of DNA to membrane).

Prehybridization

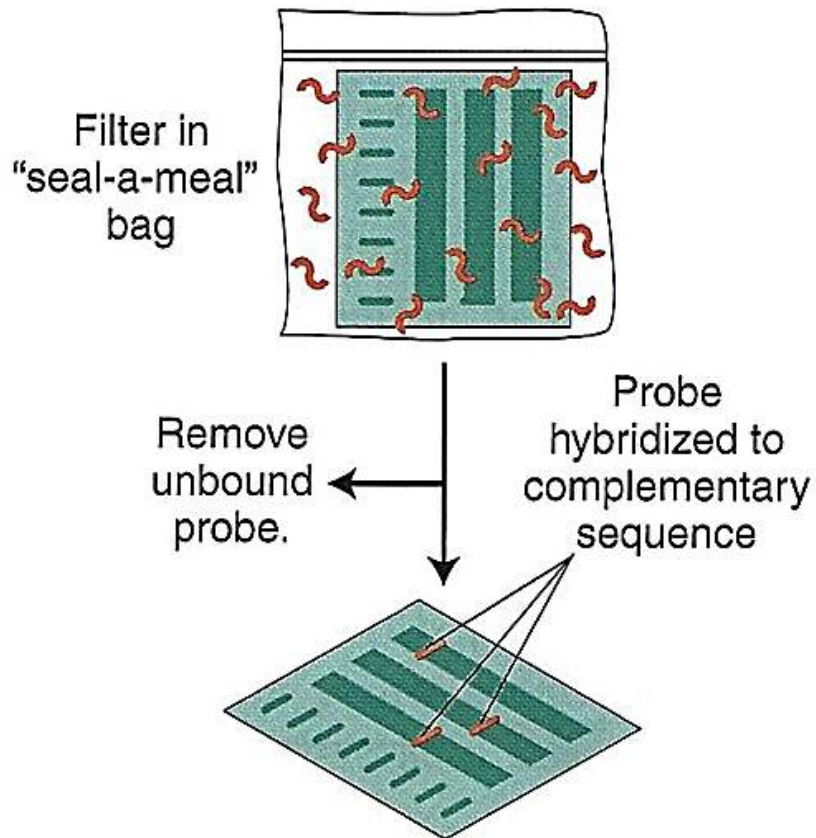
- To **block the unused DNA binding sites** on the membrane.
- Sonicated **salmon sperm DNA** is commonly used.
- Denhardt's solution containing **Ficoll** (biological polymers, a carbohydrate-based compound) and **Polyvinylpyrrolidone** (nonbiological polymeric compounds) and **BSA** (bovine serum albumen, a non-specific protein), **SDS** (sodium dodecyl sulfate), and **formamide** (destabilizing agent) are added during prehybridization process.

Membrane probing

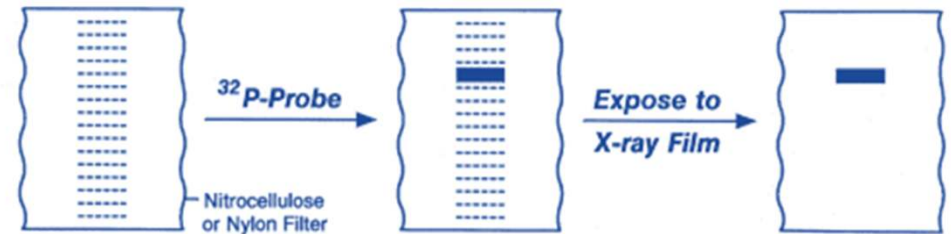
Label the probe to be hybridized using radioactive or non radioactive methods

- Radioactive probe: ^{32}P labeled ATP (Nick translation)
- Non-radioactive methods: Colorimetric
Chemiluminescent
Fluorescent

Probe hybridization



Autoradiograph



Applications of Southern blot

It can be used for identification of DNA fragment in a DNA sample

It is significantly useful in identification of criminals and is a tool in forensics

Diagnosis of Infectious disease and HIV-1

Used in personal Identification

Used in phyllogenetic analysis

Used in paternity and maternity tests

It can be used to map the restriction sites and make RFLP maps.

Used to identify mutations, deletions and gene rearrangements.

Used in prenatal diagnosis of genetic diseases (Sickle cell anaemia)



Northern Blotting

Northern blotting was developed by **Jame Alwine and George Stark at Stanford University and was named such by analogy to Southern blotting.**

Steps involved in Northern blot

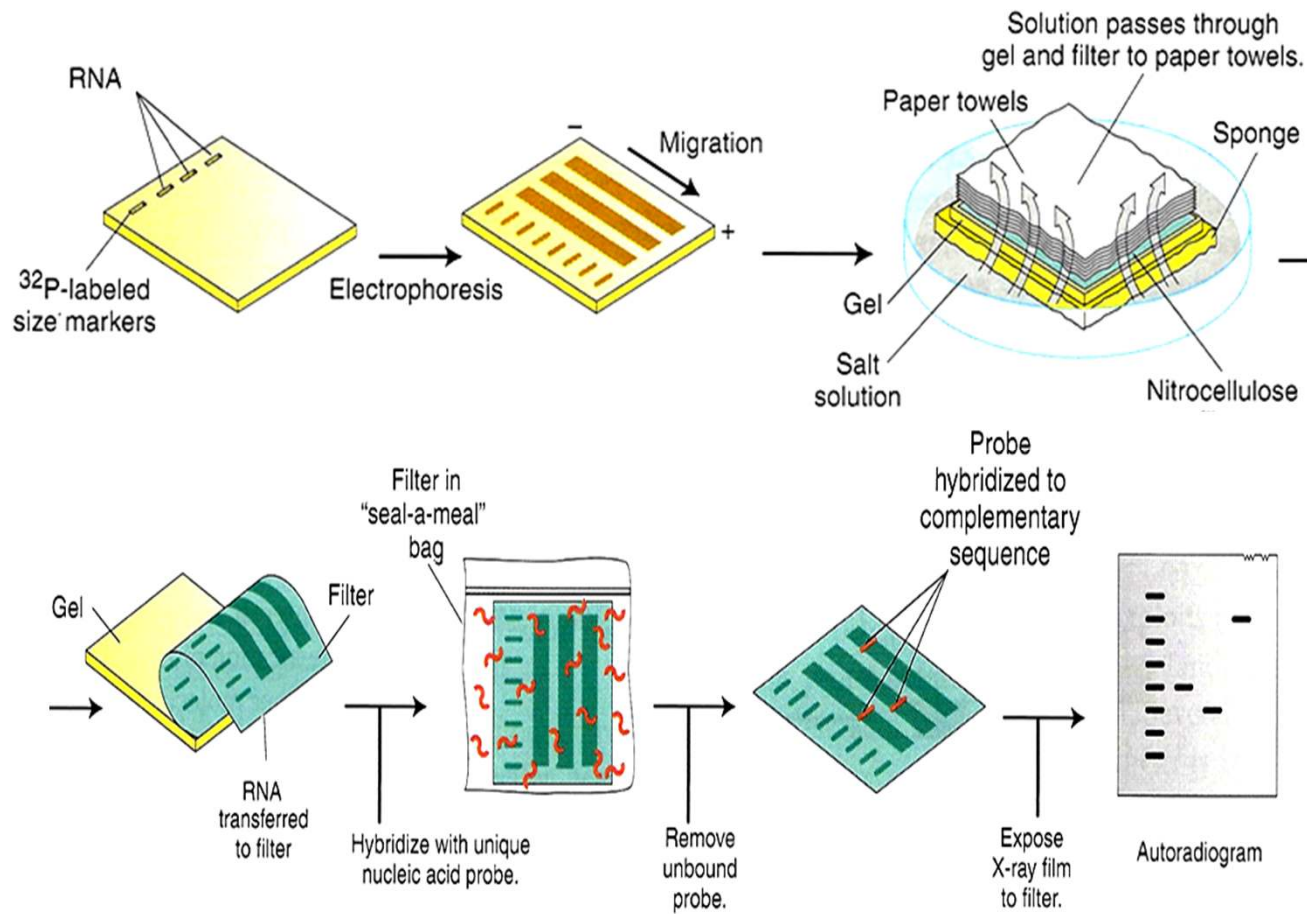
- RNA isolation
- Separation of RNA using Gel Electrophoresis
- Blotting
- Hybridization and Washing of excess probes
- Visualization

Denaturing Gel (formaldehyde or glyoxal/DMSO).

RNAse free environment.

Procedure

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Applications

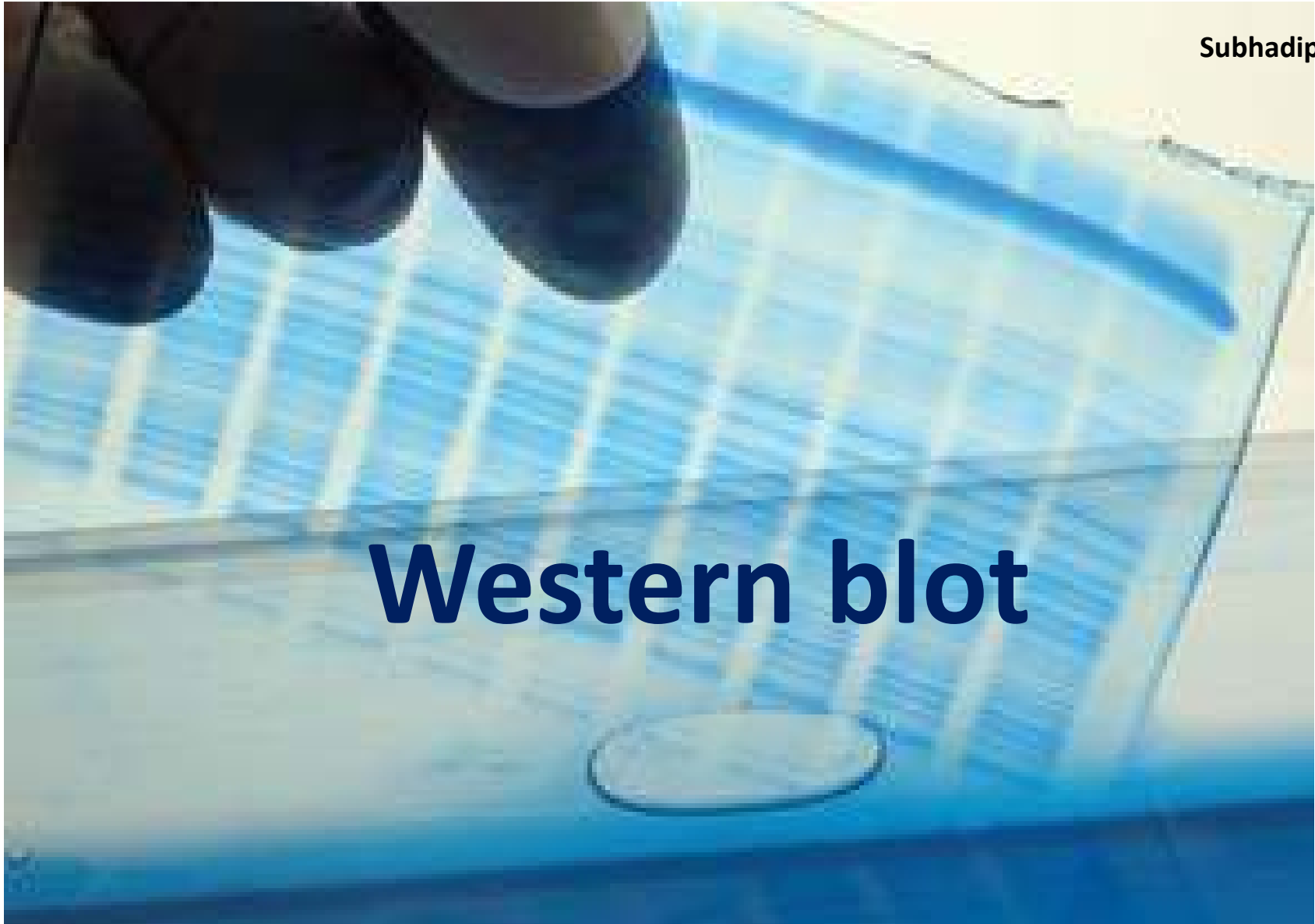
- Used to observe a particular **gene's expression** pattern between tissues, organs, developmental stages.
- Used to show **overexpression** of **oncogenes** and **downregulation of tumor-suppressor genes** in cancerous cells when **compared to 'normal' tissue**.
- It includes the detection of **RNA size**, the observation of **alternate splice products**, the use of probes with partial homology, the **quality and quantity of RNA**.

Limitations

- Method has **lower sensitivity** when compared to that of RT-PCR and nuclease protection assays.
- The technique requires **a large amount of target RNA** sample sequence.
- The northern blot technique is **very sensitive to even slight degradation** of the RNA samples. Degradation drastically affects data quality and quantitation.
- The **technique becomes laborious** in cases where multiple probes need to be added. A second probe can be added to the system only after removing the first probe completely. This can make the process highly complex. After treating the system with harsh chemicals to remove the first probe, the accuracy of the data from the subsequent rounds may also be compromised.
- Northern blot can only **measure steady state mRNA** accumulation and not RNA stability or transcription rates.

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Western blot



Steps

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Extraction of protein

Gel electrophoresis

Blotting

Blocking

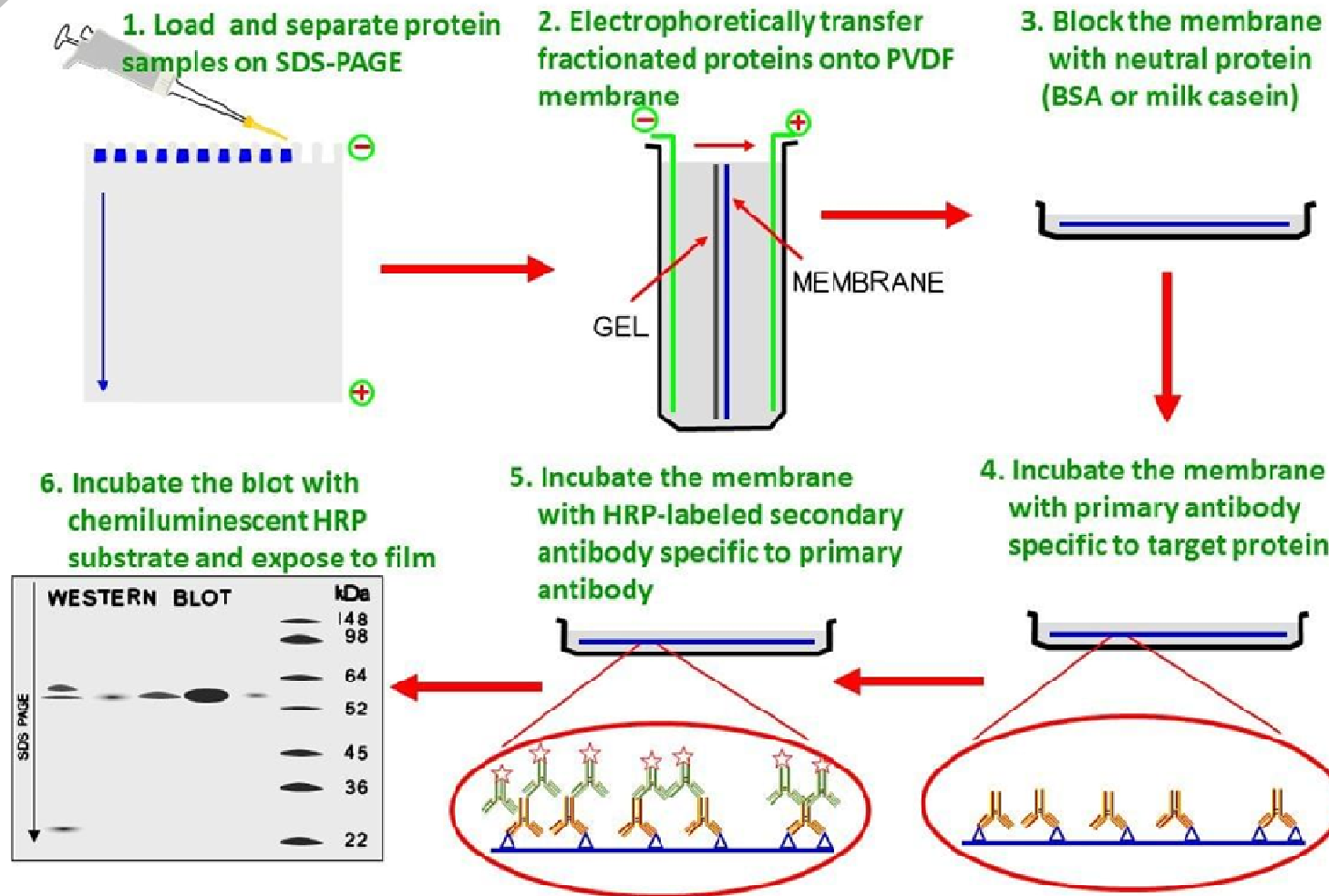
Treatment with primary antibody

Treatment with secondary antibody

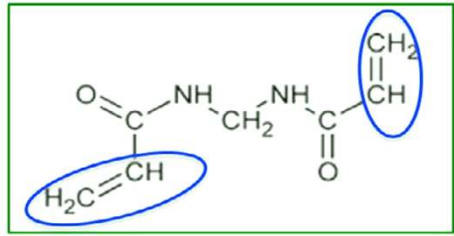
Detection and visualization

Procedure

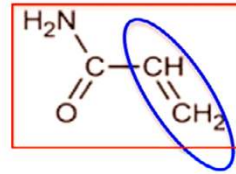
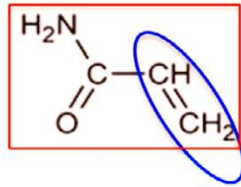
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Polymerization reaction



N,N'-methylenebisacrylamide crosslinking monomer

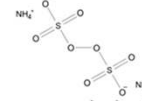


acrylamide monomer

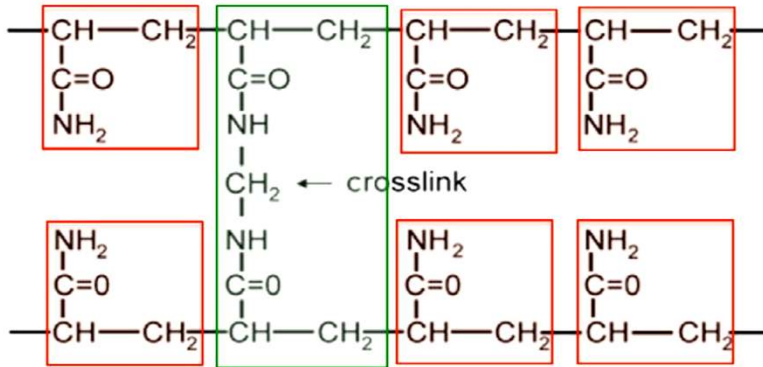
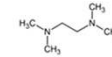


ammonium persulfate
TEMED

APS: Ammonium persulfate, radical initiator



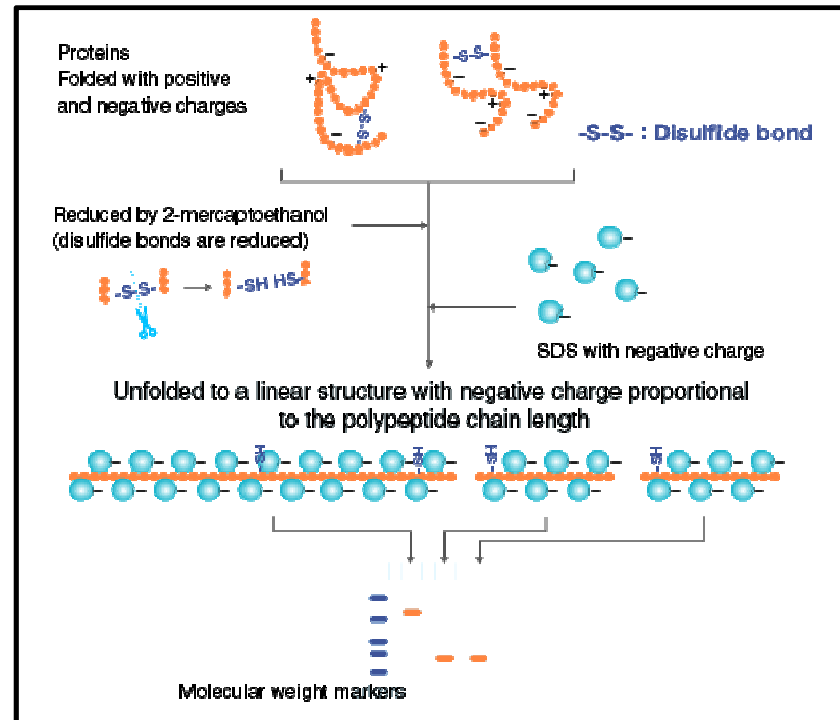
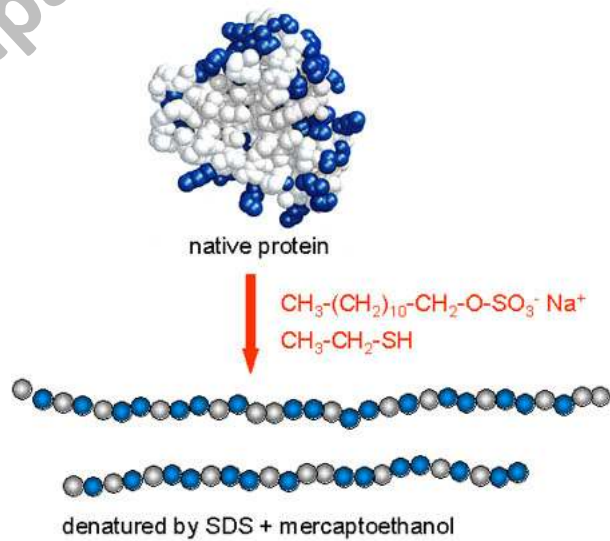
TEMED: N, N, N', N'-tetramethylethylenediamine, free radical stabilizer



polyacrylamide

Native and SDS treated protein

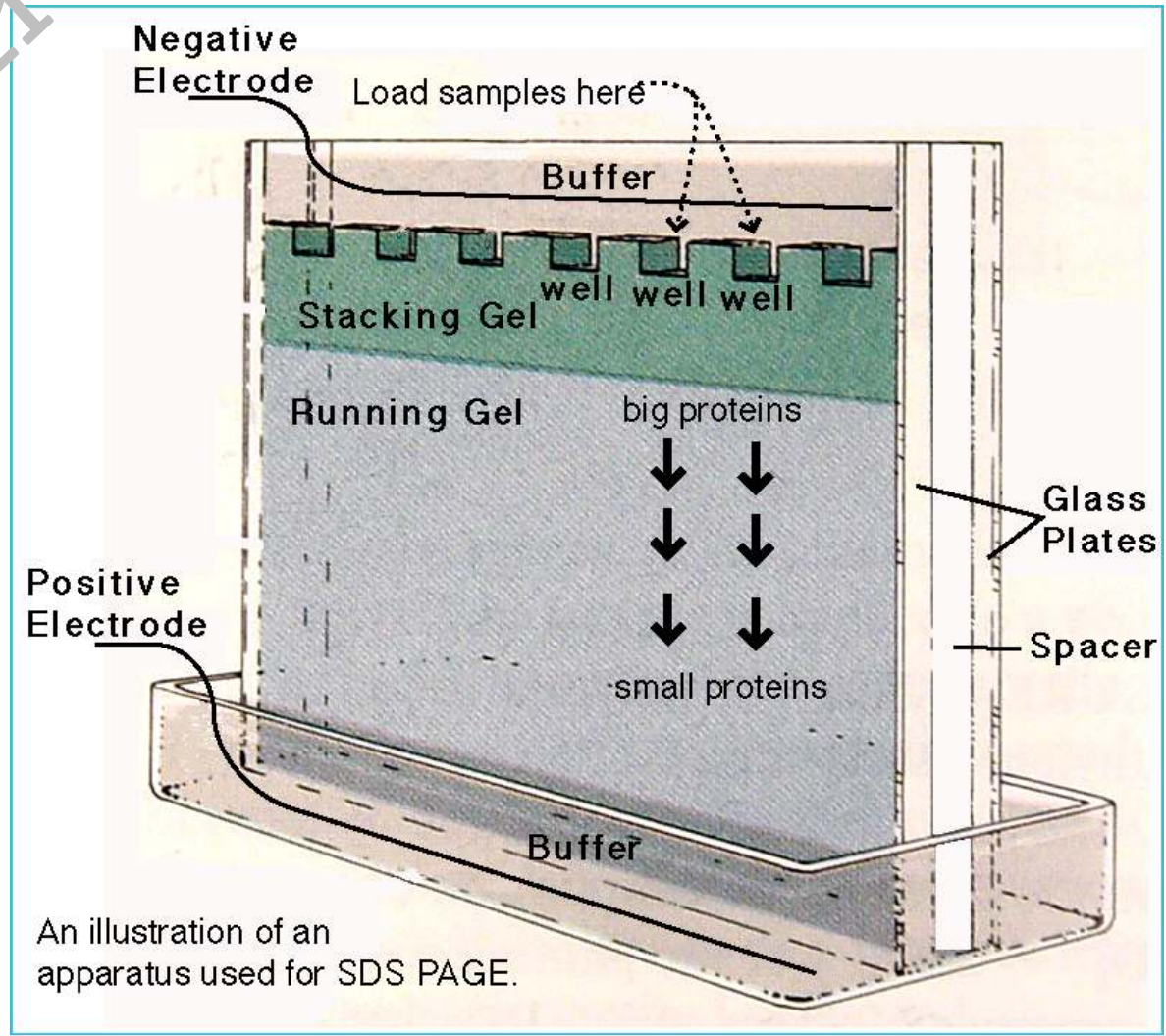
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Stacking and resolving gel

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An illustration of an apparatus used for SDS PAGE.

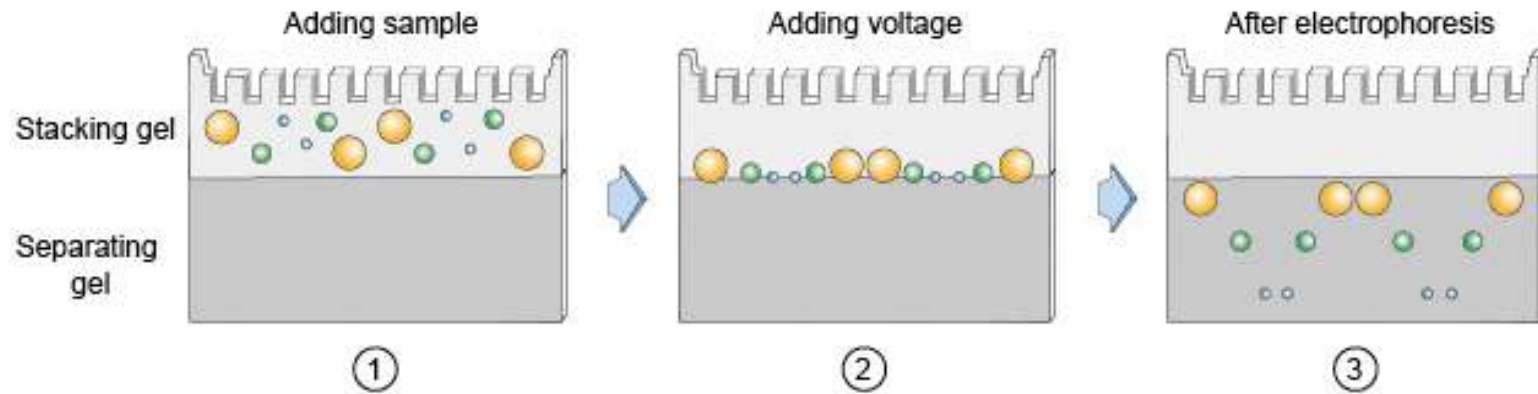
Sample run

Polymerized gel:

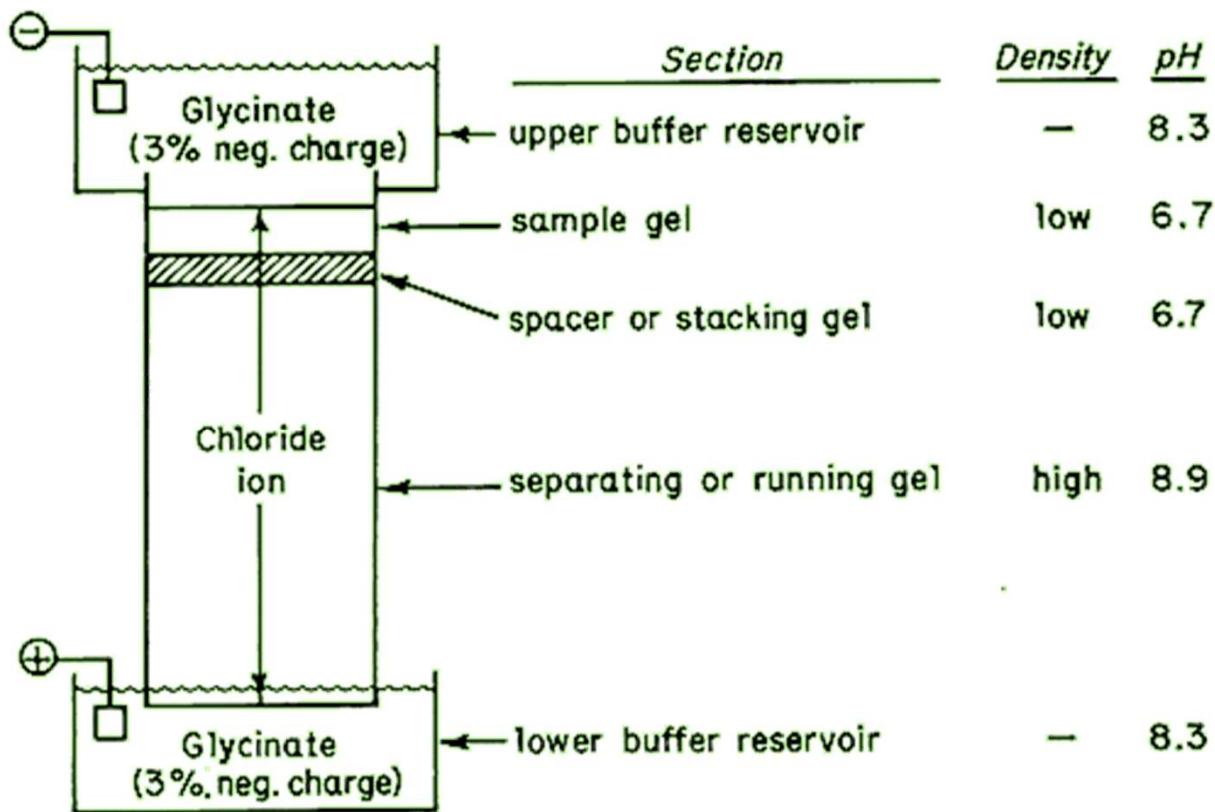
- Resolving gels made in 6%, 10%, 12%, 18%.
- Stacking Gel up to 5% was added to gel and then the wells are created.

The percentage chosen **depends on the size of the protein** that one wishes to identify or probe in the sample.

The smaller it is the bigger the percentage



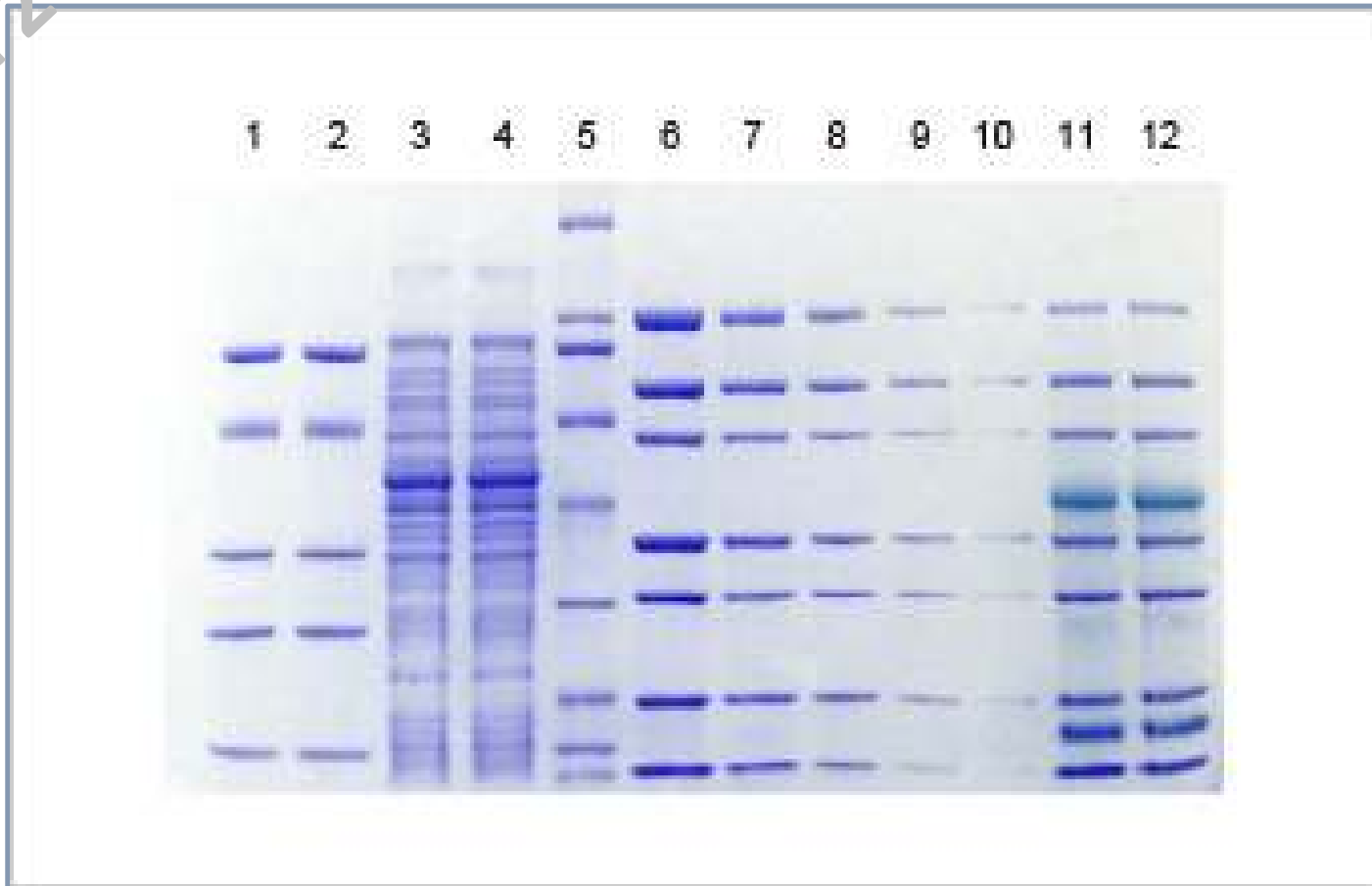
pH at different sites



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Protein gel

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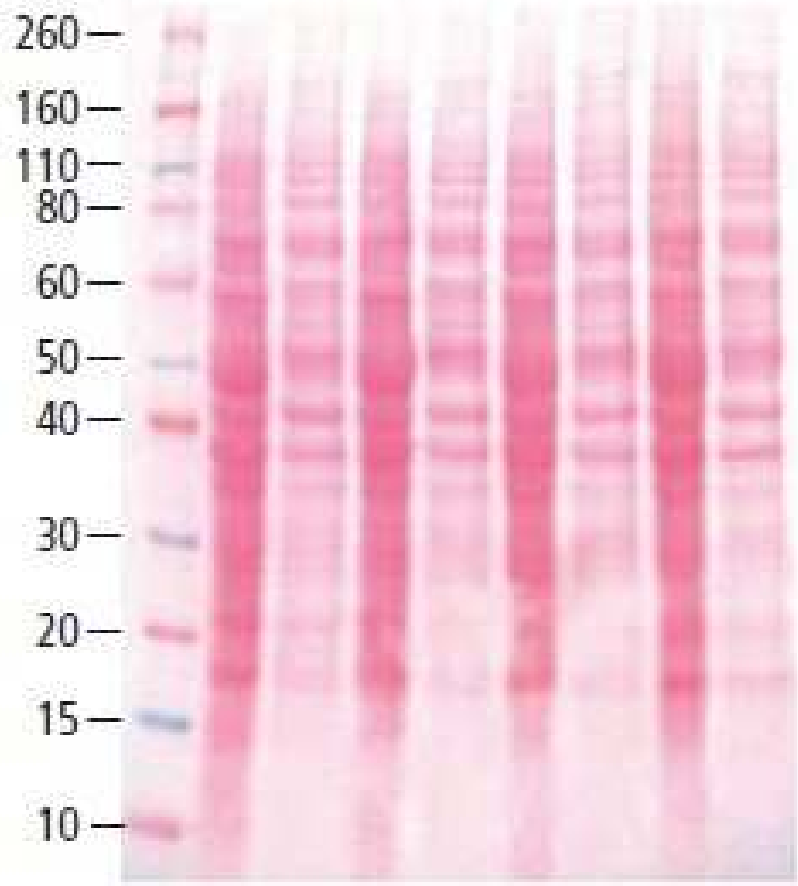


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Ponceau staining

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Mr (kDa)



PVDF membrane

(Methanol activation: It allows improved transfer and protein binding)



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Western blot setup

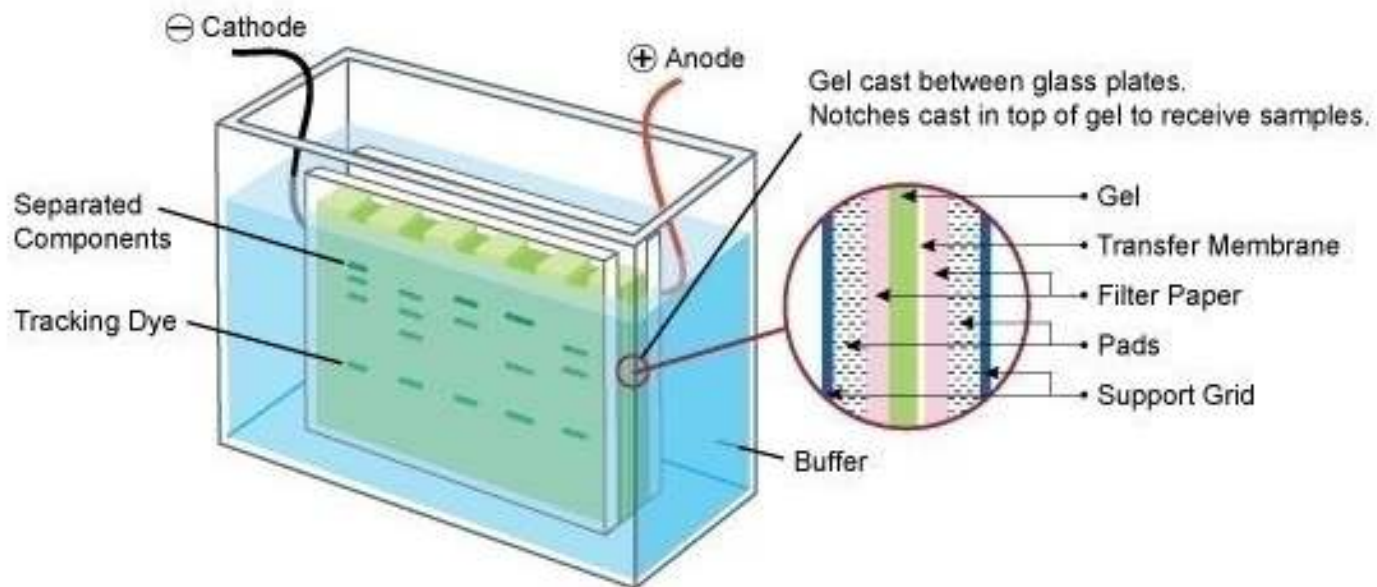


Diagram 1: Illustration of Western Blot Setup.

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Wet transfer machine

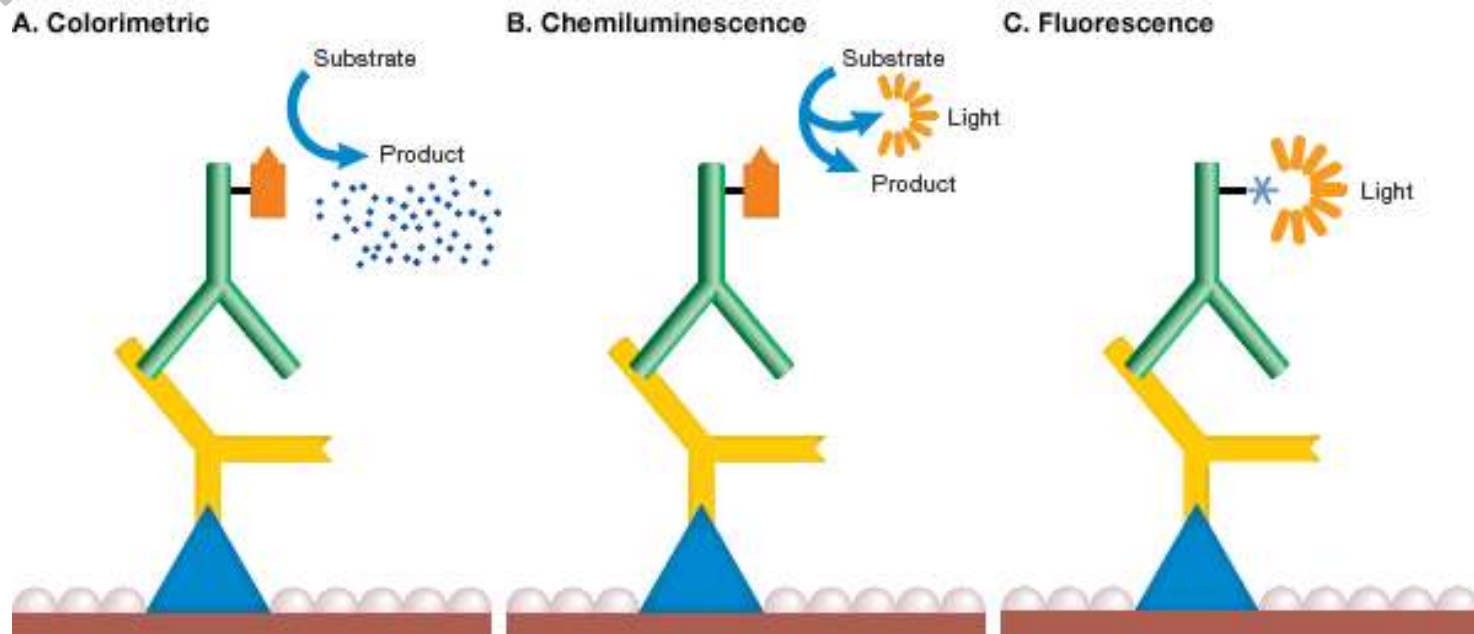


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Semi-dry transfer

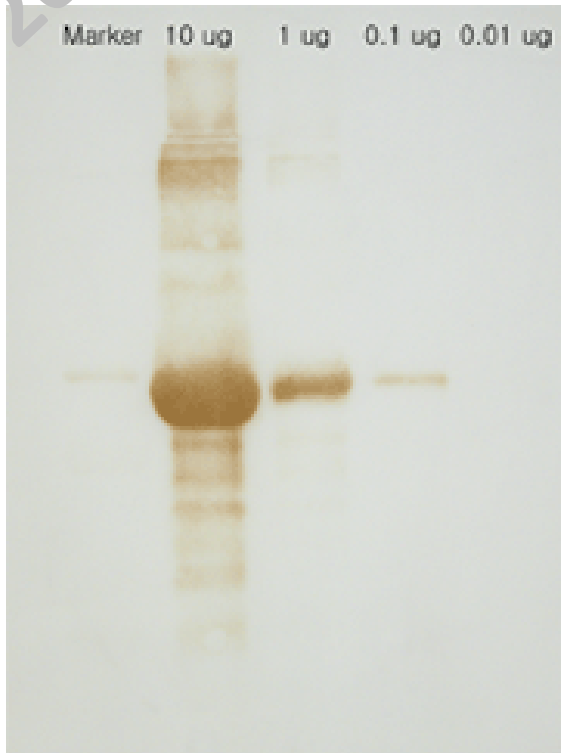


Detection methods



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Visualization



Applications of Western blot

1. To determine the size and amount of protein in given sample.
2. Disease diagnosis: detects antibody against virus or bacteria in serum.
3. Western blotting technique is the confirmatory test for HIV.
4. Useful to detect defective proteins (Prions disease).
5. Definitive test for Creutzfeldt-Jacob disease, Lyme disease, Hepatitis B and Herpes.