



Blotting Techniques

(Southern blot, Northern blot, Western blot, and Eastern blot)



Learning Objectives:

- ▷ What is blotting?
- ▷ Blotting Techniques Types.
- ▷ Applications for each technique.
- ▷ Practical part (Western Blotting Virtual Lab).

Key Terms



Complementary DNA (cDNA): DNA created *in-vitro* by using reverse transcriptase to synthesize DNA from mRNA templates.



DNA probe:

A short, labelled, single strand of DNA or RNA used to locate its complementary strand in a quantity of DNA.



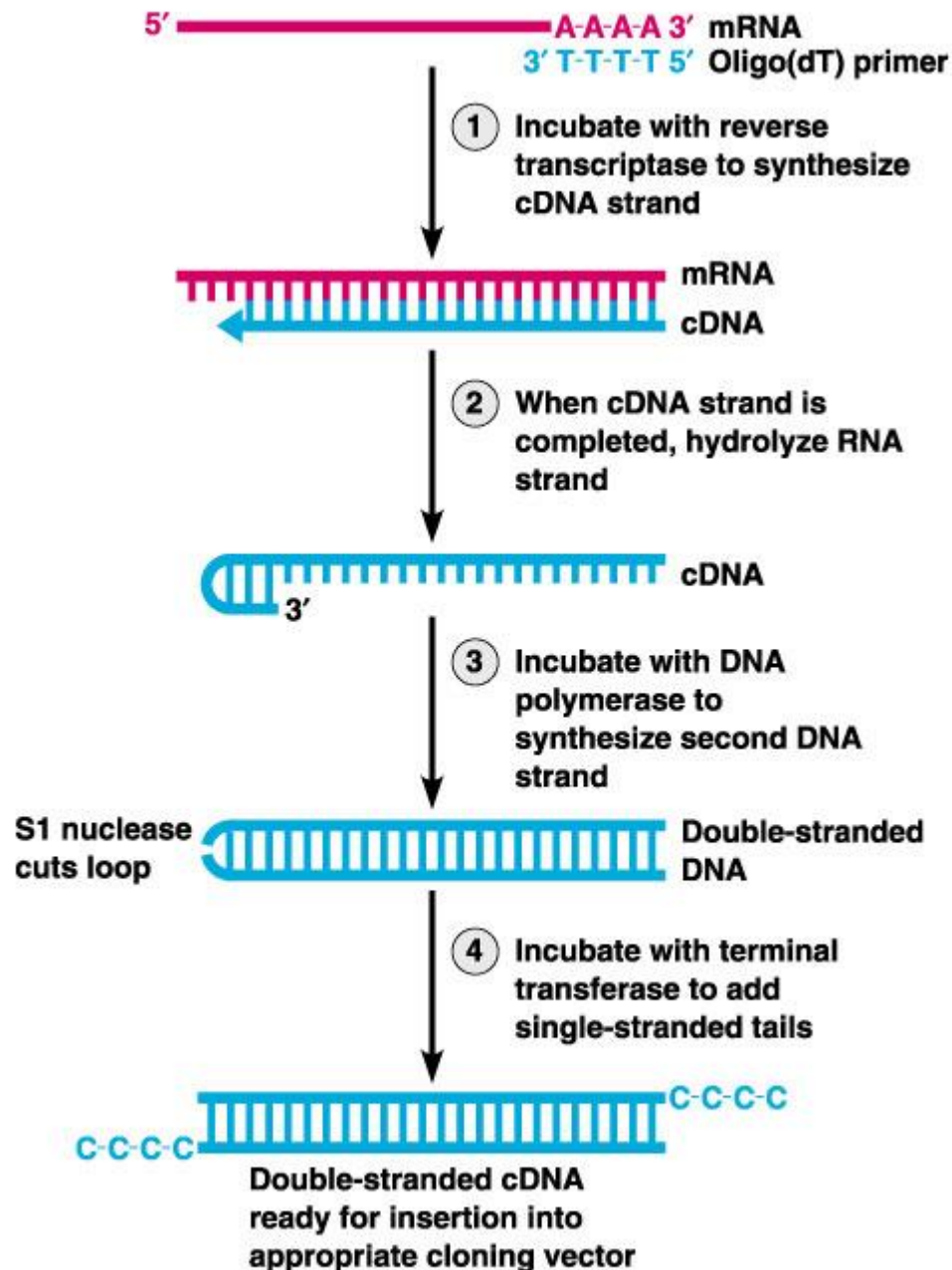
Hybridization:

Process of forming a dsDNA molecule between a ssDNA probe and a target ssDNA



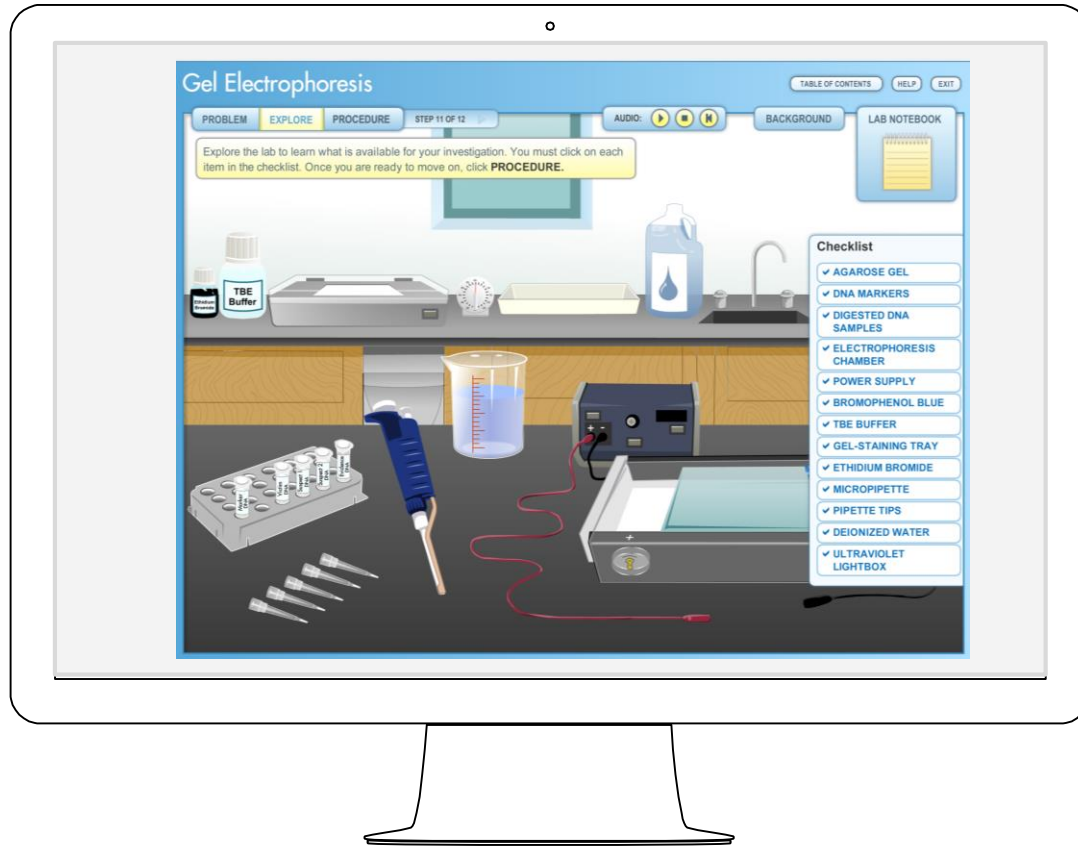
Gel electrophoresis:

The separation of substances (such as serum proteins or DNA) by their rate of movement through an electrical field.



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Figure 1. Preparation of Complementary DNA (cDNA).

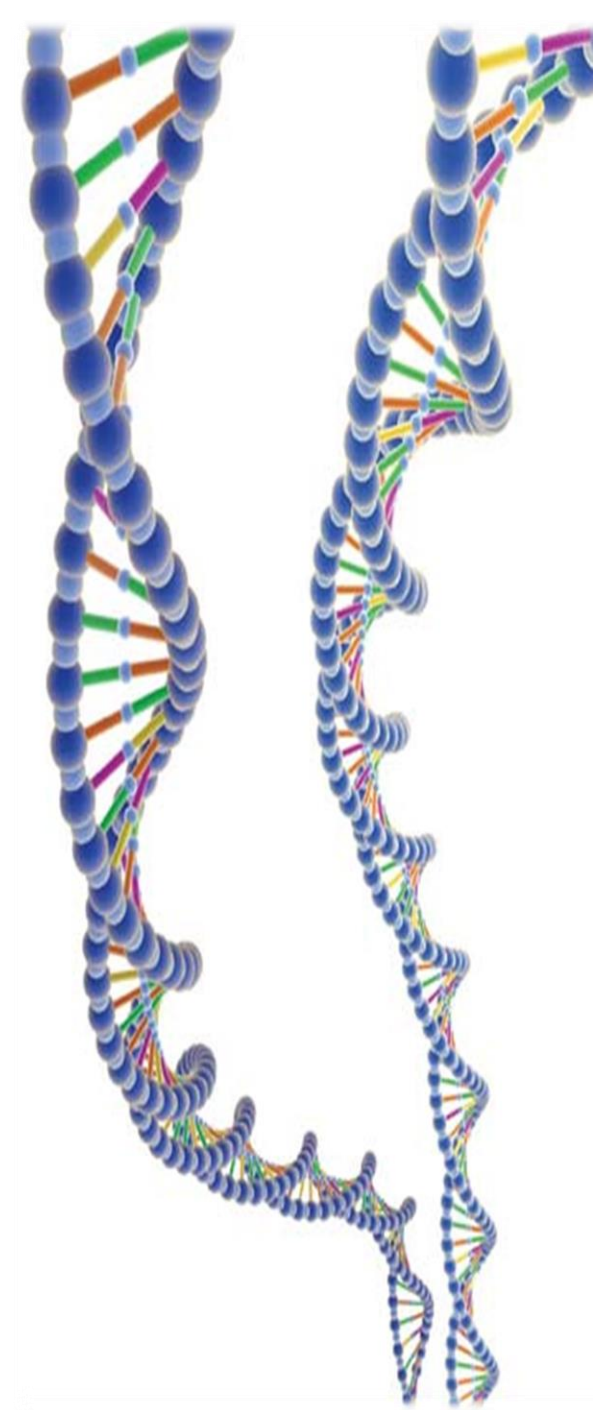


Gel electrophoresis (Virtual Lab)

http://www.classzone.com/books/hs/ca/sc/bio_07/virtual_labs/virtualLabs.html

What is blotting?

Blots are techniques for transferring **DNA**, **RNA** and **proteins** onto a carrier so they can be separated, and often follows the use of a gel electrophoresis. The **Southern blot** is used for transferring **DNA**, the **Northern blot** for **RNA** and the **Western blot** for **Protein**.



Blotting Techniques Types

Eastern blot

For detecting protein post transnational modifications (PTM)

Western blot

For detecting Protein

Southern blot

For detecting DNA

Northern blot

For detecting RNA

1.

SOUTHERN BLOTTING

1975



Professor **Sir Edwin Southern**, developed this method in 1975.

Southern won the **Lasker Award for Clinical Medical Research** prize for the method of finding specific **DNA** sequences.

The technique is known as DNA transfer or '**Southern blot**'

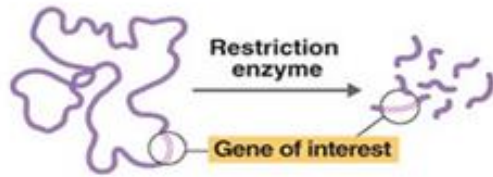


Principle

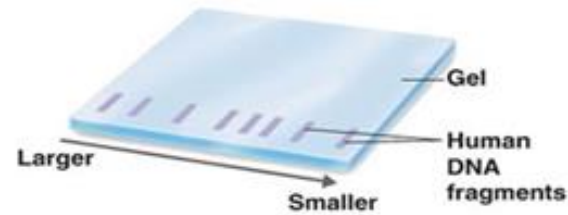
▷It is a method routinely used in molecular biology for detection of a specific DNA sequence in DNA samples. The DNA detected can be a **single gene**, or it can be part of a **larger piece of DNA** such as a viral genome.

▷Southern blotting combines **agarose gel electrophoresis** for size separation of DNA with methods to **transfer** the size separated DNA to a filter membrane for **probe hybridization**.

Steps in southern blotting



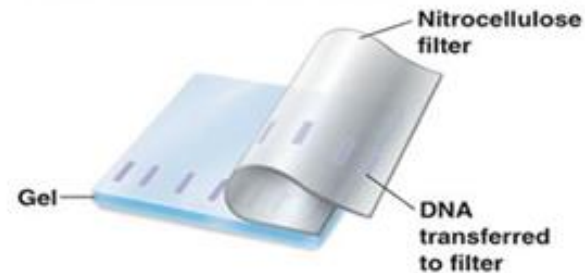
- 1 DNA containing the gene of interest is extracted from human cells and cut into fragments by restriction enzymes.



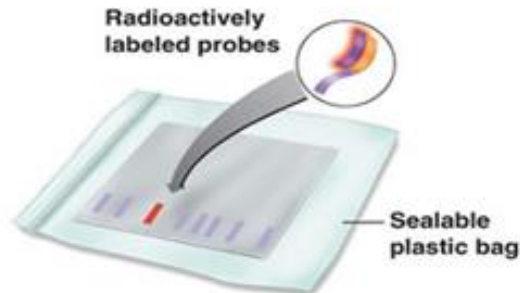
- 2 The fragments are separated according to size by gel electrophoresis. Each band consists of many copies of a particular DNA fragment. The bands are invisible but can be made visible by staining.



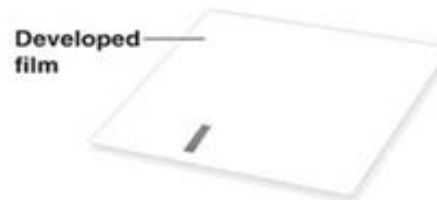
- 3 The DNA bands are transferred to a nitrocellulose filter by blotting. The solution passes through the gel and filter to the paper towels.



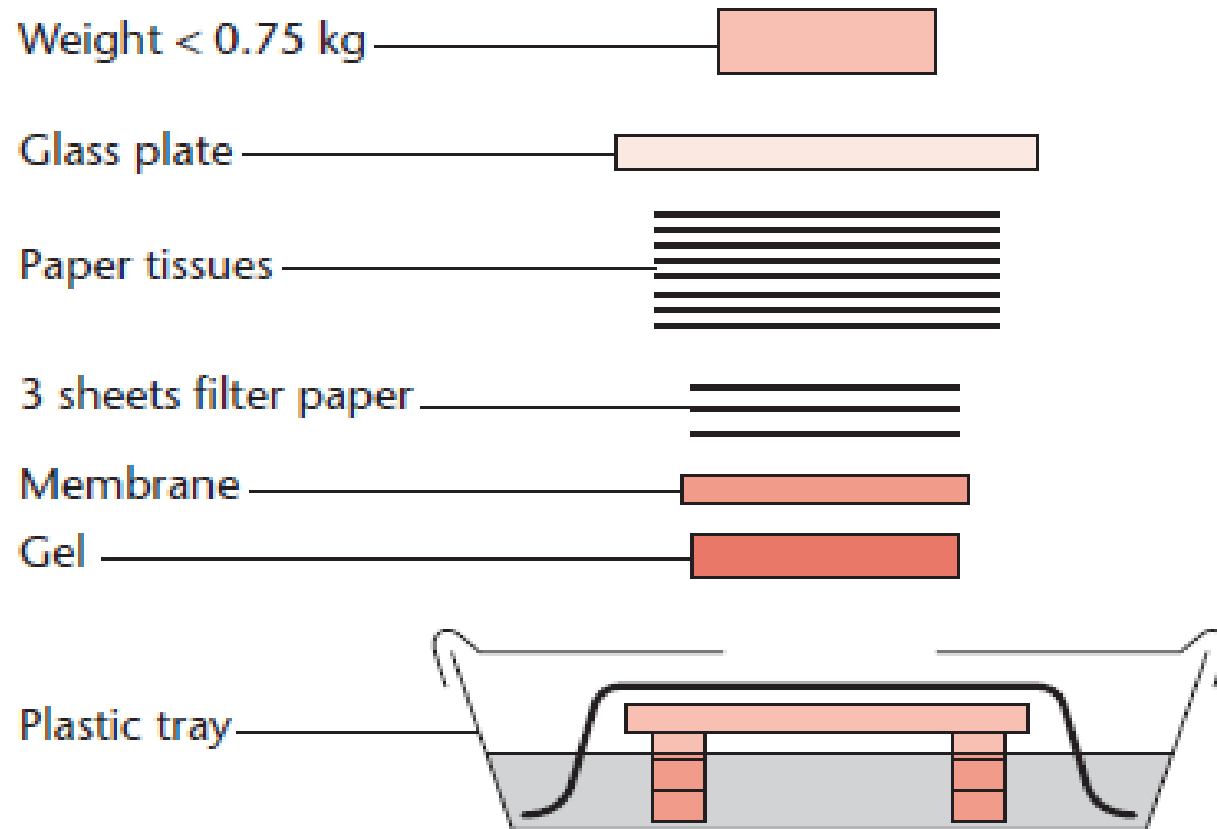
- 4 This produces a nitrocellulose filter with DNA fragments positioned exactly as on the gel.



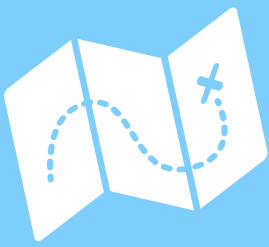
- 5 The filter is exposed to a radioactively labeled probe for a specific gene. The probe will base-pair (hybridize) with a short sequence present on the gene.



- 6 The filter is then exposed to X-ray film. The fragment containing the gene of interest is identified by a band on the developed film.



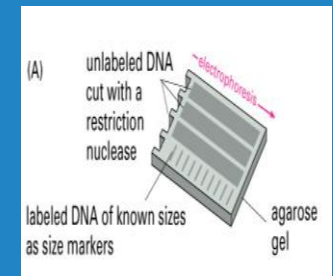
Capillary blotting apparatus



Steps in southern blotting

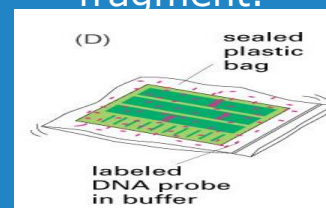
1. Digest the DNA with an appropriate restriction enzyme.

2. The complex mixture of fragments is subjected to gel electrophoresis to separate the fragments according to size.

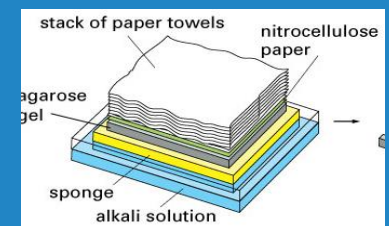
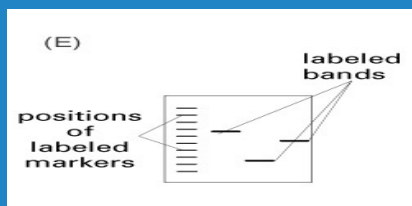


3. The restriction fragments present in the gel are denatured with alkali and transferred onto a nitrocellulose filter or nylon membrane by blotting.

4. The filter is incubated under hybridization conditions with a specific radiolabeled DNA probe. The probe hybridizes to the complementary DNA restriction fragment.



5. Excess probe is washed away and the probe bound to the filter is detected by autoradiography, which reveals the DNA fragment to which the probe hybridized.





APPLICATIONS

▷ gene discovery , mapping, evolution and development studies, diagnostics and forensics.

▷ identification of the transferred genes in transgenic individuals, etc.

▷ investigators 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.

2.

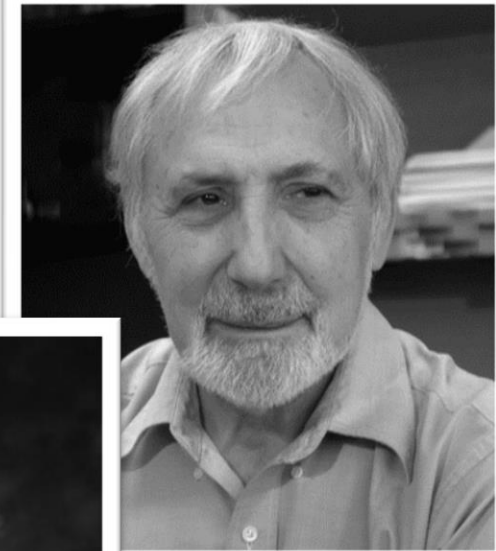
NORTHERN BLOTTING

1979



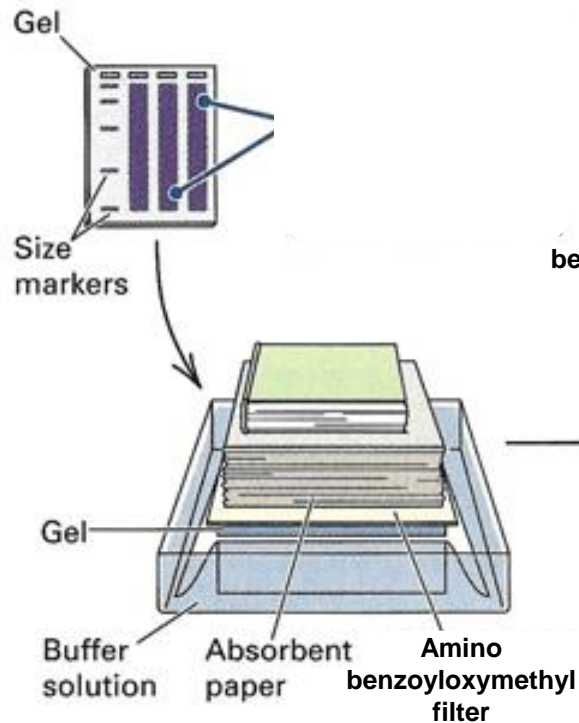
Northern blotting is a technique for detection of **specific RNA sequences**.

Northern blotting was developed by **James Alwine and George Stark** at Stanford University **1979** and was named such by analogy to Southern blotting

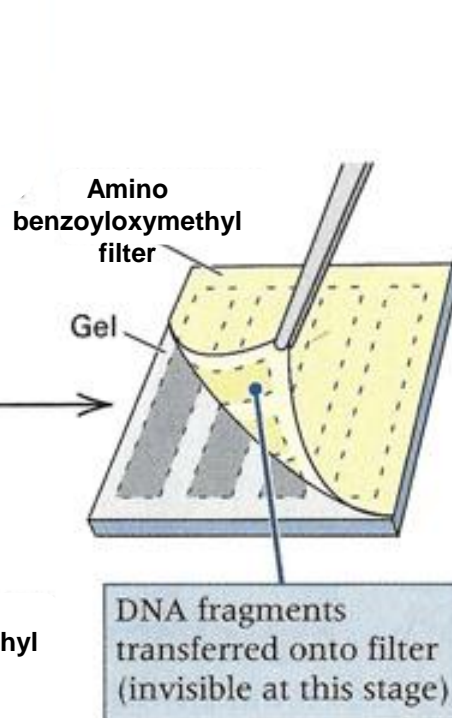


Northern blot

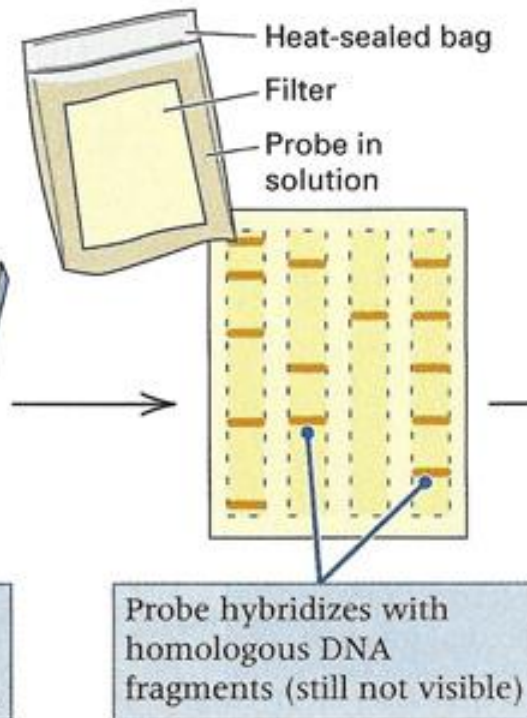
(A) RNA is cleaved; electrophoresis is used to separate RNA



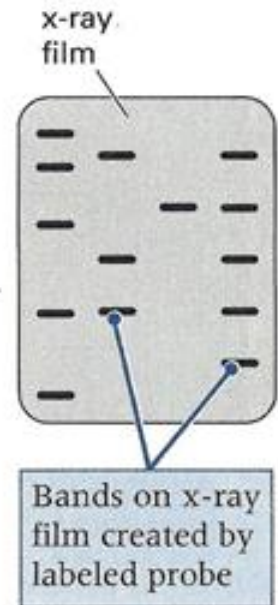
(B) RNA fragments are blotted onto Amino benzoyloxymethyl filter

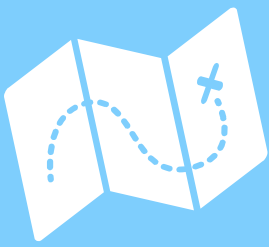


(C) Filter is exposed to radioactive probe



(D) Filter is exposed to photographic film; film is developed

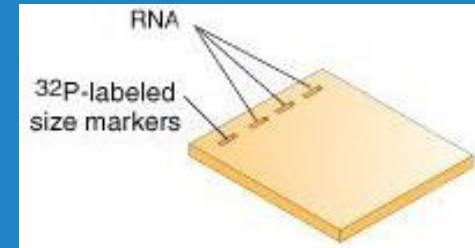




Steps in Northern blotting

1. RNA is isolated from several biological samples (e.g. various tissues, various developmental stages of same tissue etc.)

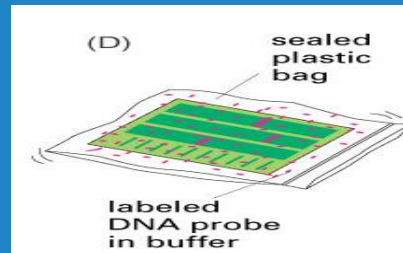
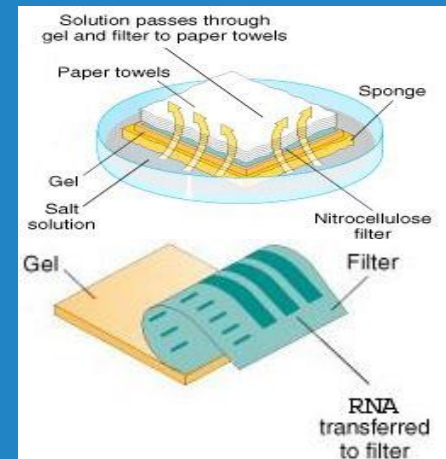
2. Samples are loaded on gel and the RNA samples are separated according to their size on an agarose gel



5. The membrane is washed to remove unbound probe. The labeled probe is detected via autoradiography or via a chemiluminescence reaction (if a chemically labeled probe is used). In both cases this results in the formation of a dark band on an X-ray film.

4. The membrane is placed in a dish containing hybridization buffer with a labeled probe.

3. The gel is then blotted on a nylon membrane or a **Amino benzoyloxymethyl filter paper** by creating the sandwich arrangement.





APPLICATIONS

Detect the expression level (mRNA) and transcript size of a specific gene in a specific tissue or at a specific time. Sometimes mutations do not affect coding regions but transcriptional regulatory sequences (e.g., promoter, splice sites, copy number, transcript stability)

Disadvantage of Northern blotting

1. The standard northern blot method is relatively less sensitive than nuclease protection assays and RT-PCR
2. Detection with multiple probes is a problem
3. If RNA samples are even slightly degraded by RNAses, the quality of the data and quantitation of expression is quite negatively affected.
4. Use of radioactivity(although non-radioactive techniques are available)- Laborious if many genes need to be tested- Assay is time-consuming.

3.

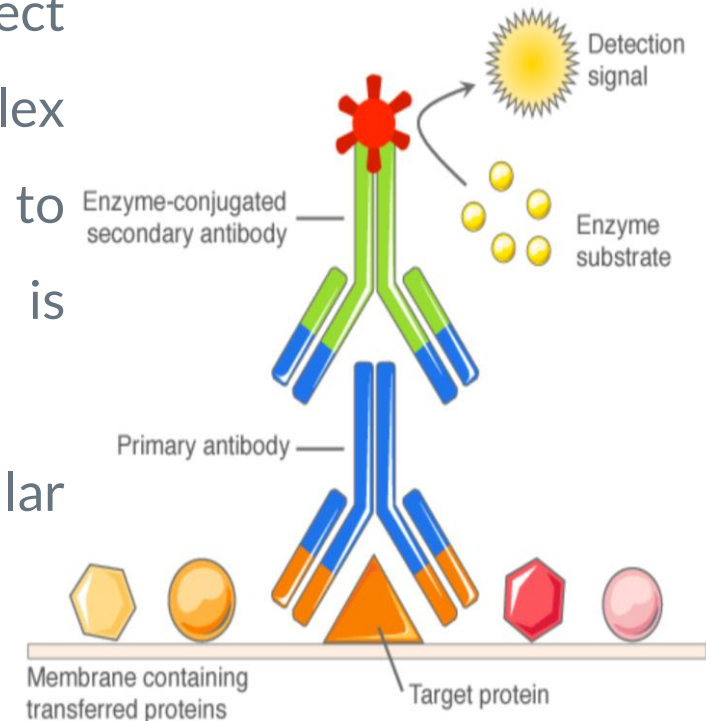
WESTERN BLOTTING

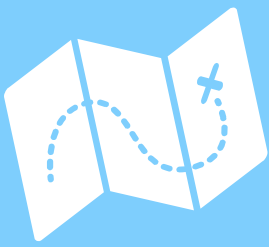
1981



Western blotting, also known as **immunoblotting** or **protein blotting**, is a technique used to detect the presence of a specific protein in a complex protein mixture according to their **size** and **amount**. In other words, WB is used to determine '**protein expression**'.

It is a core technique in cell biology, molecular biology, virology and others





Steps in Western blotting

Sample Prep



Separate Proteins



Transfer proteins to membrane



Block membrane



1° antibody



Wash



2° antibody

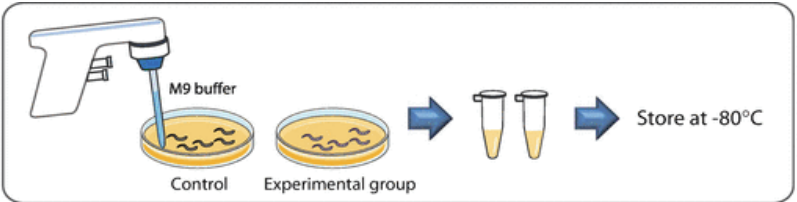


Wash

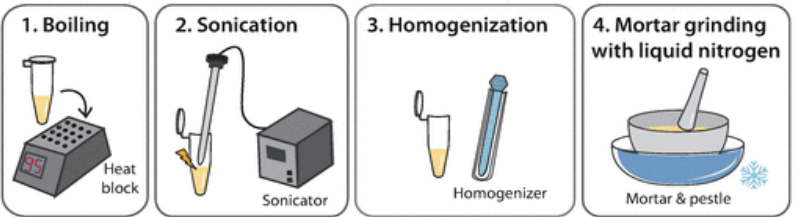


Detection

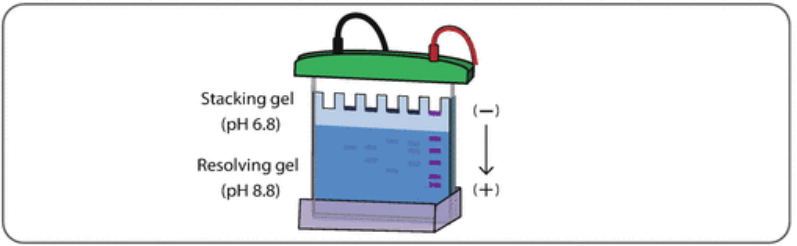
1. Preparation of samples



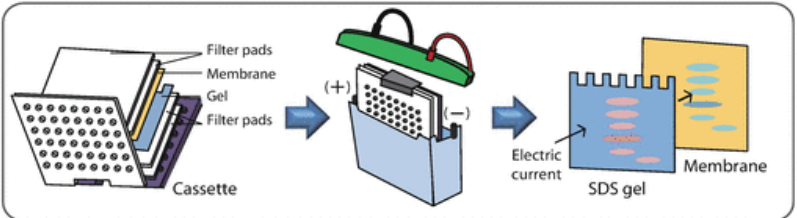
2. Protein extraction



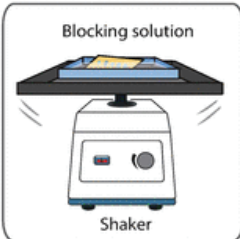
3. SDS-PAGE



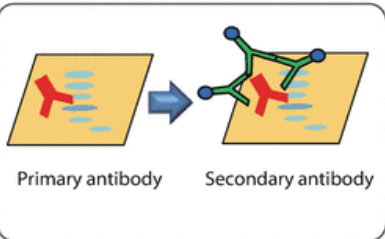
4. Protein transfer



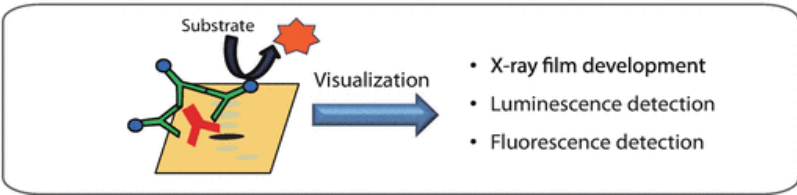
5. Blocking the membrane



6. Antibody incubation



7. Detection



Lysis depends on tissue:
Culture Cells -> sonicate
Tissue samples -> homogenise
*Centrifuge to remove debris.
*Keep cold and use protease inhibitors and phosphatase inhibitors.

*Separation of proteins according to molecular weight
*Proteins are denatured before SDS-PAGE.

Transfer separated proteins onto a membrane, which can then be probed with antibodies to detect the protein of interest.
Membrane can be Nitrocellulose or PVDF

Fill up the space on the membrane to prevent non-specific antibody binding
block buffers:
Milk or BSA



APPLICATIONS

▷ For HIV

confirmatory HIV-test to detect anti-HIV antibody in a human serum sample

▷ For HBV

confirmatory test for Hepatitis B infection

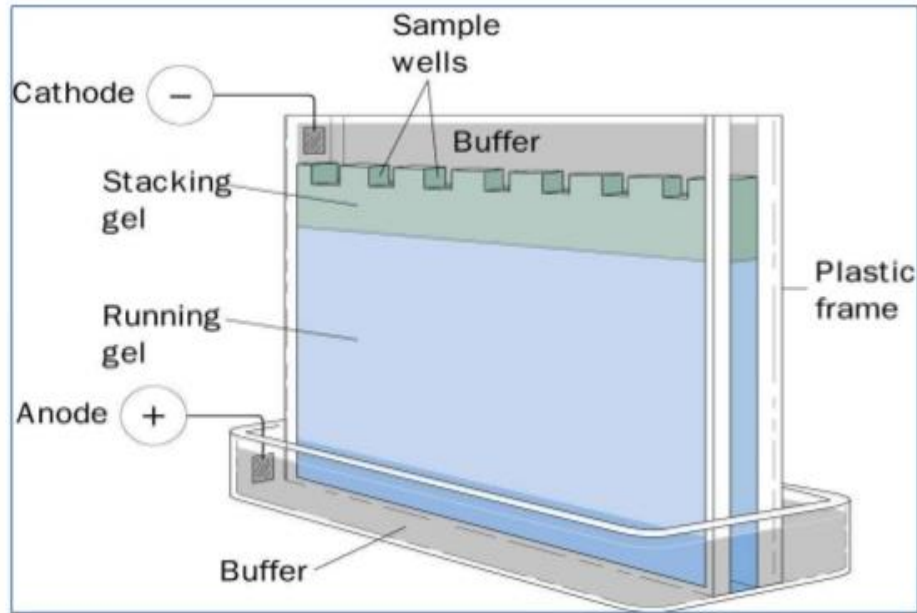
▷ For Herpes

detection of HSV infections

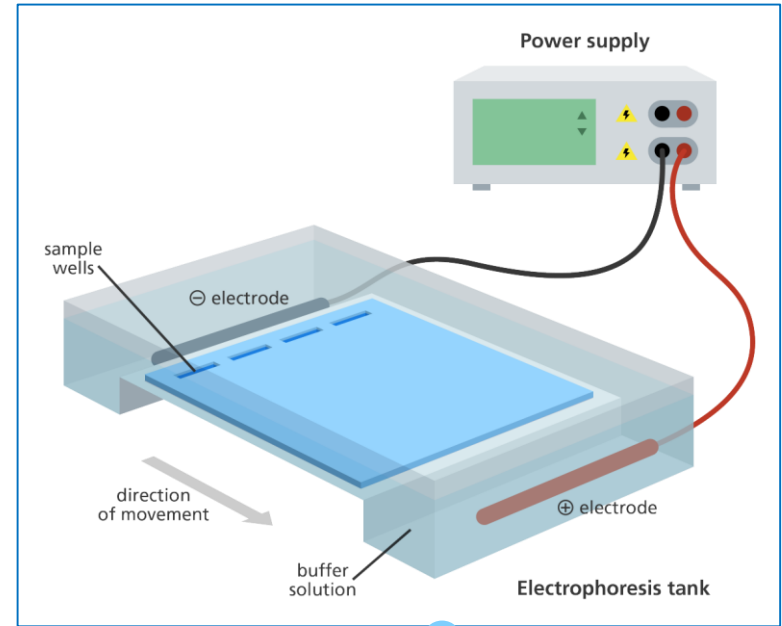
▷ A definitive test for Bovine spongiform encephalopathy (BSE)

▷ Some forms of Lyme disease testing employ Western blotting.

Vertical vs. horizontal gel electrophoresis unit



Western blot



Southern blot
and
Northern blot



Western Blotting Virtual Lab

<https://aelp.smartsparrow.com/v/open/zwzesp>

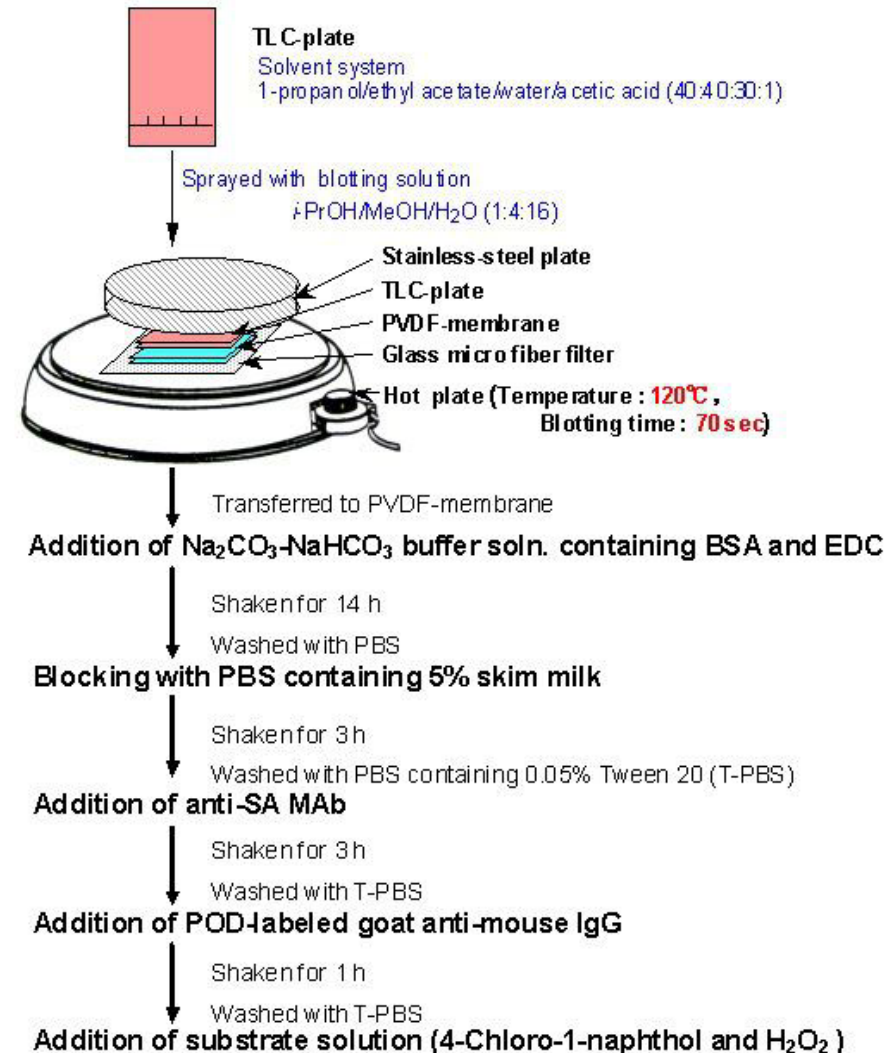
n2

3.

EASTERN BLOTTING

Eastern blotting

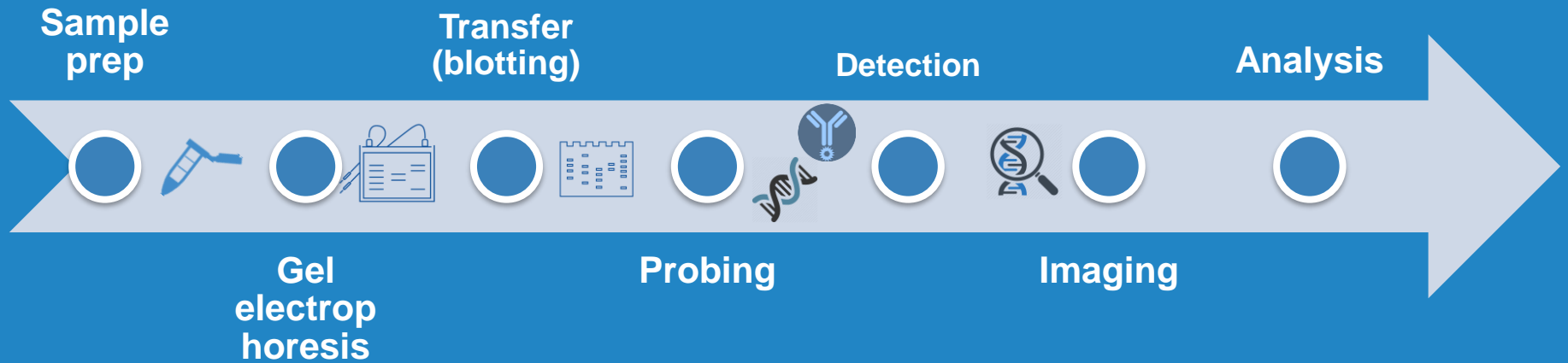
is a biochemical technique used to analyze **protein post translational modifications (PTM)** such as lipids, phosphomoieties and glycoconjugates. It is most often used to detect carbohydrate epitopes. Thus, Eastern blotting can be considered an extension of the biochemical technique of Western blotting.



Comparison of Southern, Northern, and Western blotting techniques

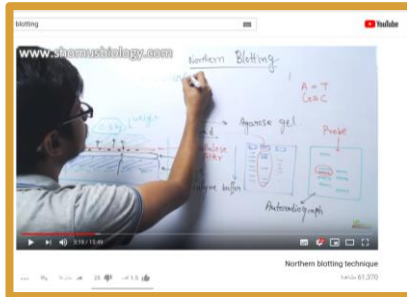
	Southern blotting	Northern blotting	Western blotting
Molecule detected	DNA (ds)	mRNA (ss)	Protein
Gel electrophoresis	Agarose gel	Formaldehyde agarose gel	Polyacrylamide gel
Gel pretreatment	Depurination, denaturation, and neutralization	-	-
Blotting method	Capillary transfer	Capillary transfer	Electric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

To sum up:





Further information



Northern blot

<https://www.youtube.com/watch?v=GBmFaf2krYY>



Eastern blot

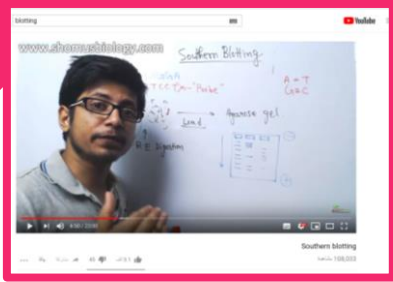
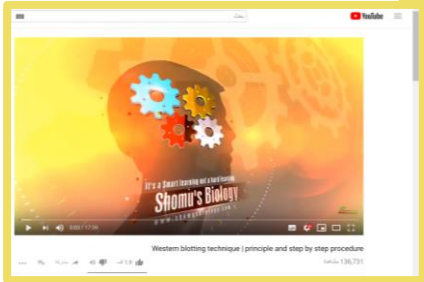
<https://www.youtube.com/watch?v=138L1663380>

Western blot

<https://www.youtube.com/watch?v=N8XbauUsns4&t=10s>

Southern blot

<https://www.youtube.com/watch?v=KVyP8v8q8NU>





SN ❄️ W

DR 💧 P

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

Training | Abcam

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د Glass Lu عمادة التعاملات الإلكترونية التحقق من السرقة العا. General Authority for Amlan Internation

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Discover your best work

Western blot

Straight forward blotting. Work through essential skills and then move on to optimization and more advanced techniques.

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“Self-education is, I firmly believe, the *only* kind of education there is.”

— Isaac Asimov

<http://go.myabcam.com/training?elqTrackId=97fbc4694f0341e3846eb99d1176c809&elq=f941c791458149c69503e71c7d3f4415&elqaid=6026&elqat=1&elqCampaignId=>



Assignment



▷ Chose one of the blotting techniques discussed today (Southern blot, Northern blot, and Western blot) and write a procedure summary flow chart.

Thanks!

Any questions?



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