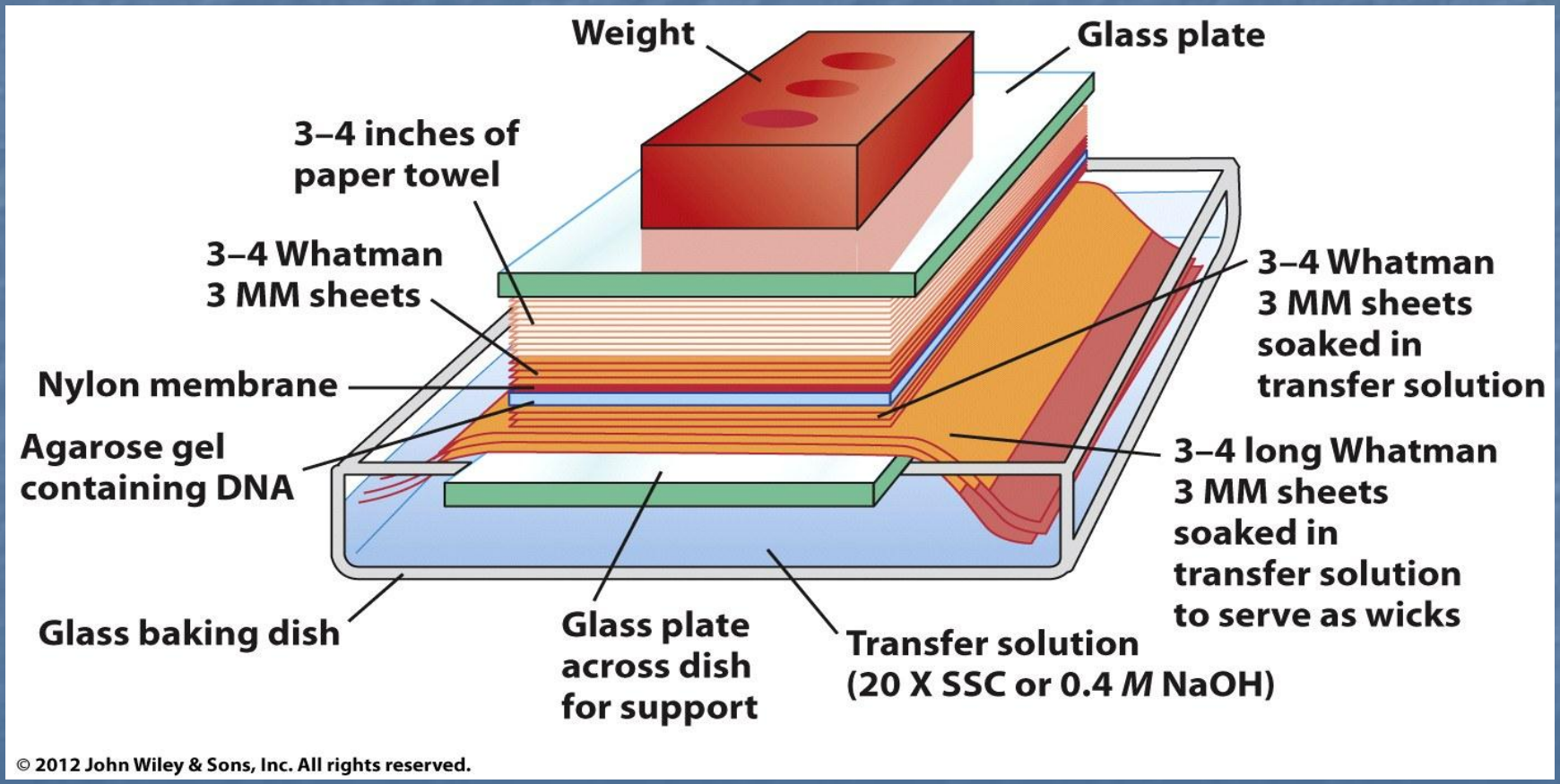
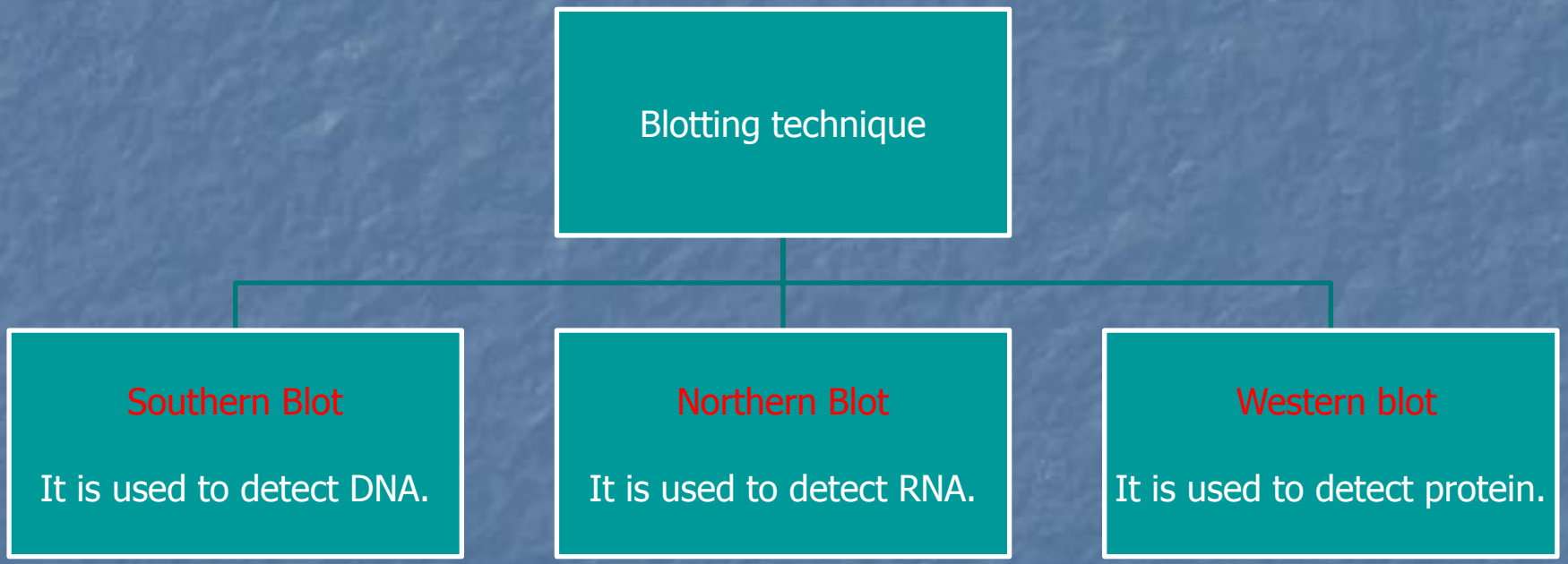
What is blotting?

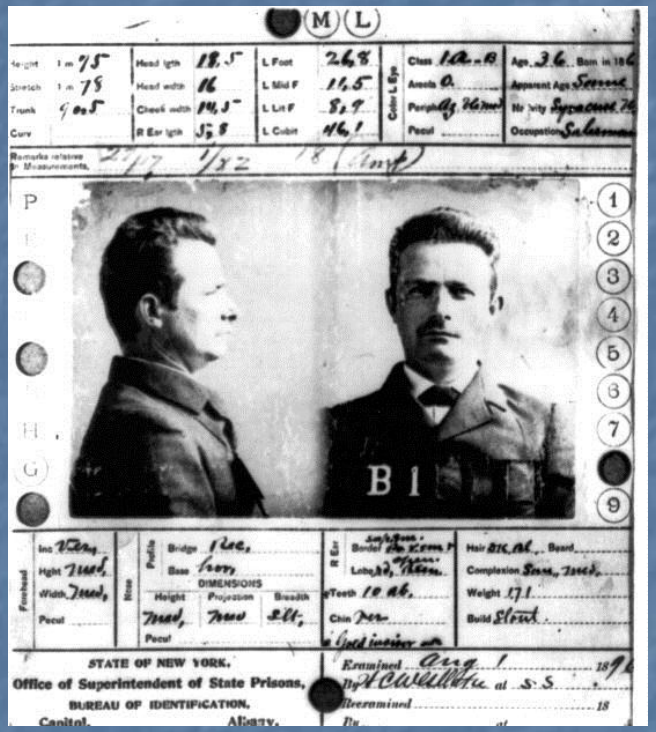
- **Blots are techniques for transferring DNA , RNA and proteins onto a carrier so they can be separated, and often follows the use of a gel electrophoresis.**



TYPES OF BLOTTING TECHNIQUES



DNA Evidence



First criminal identification card filed by the NY State Bertillon Bureau

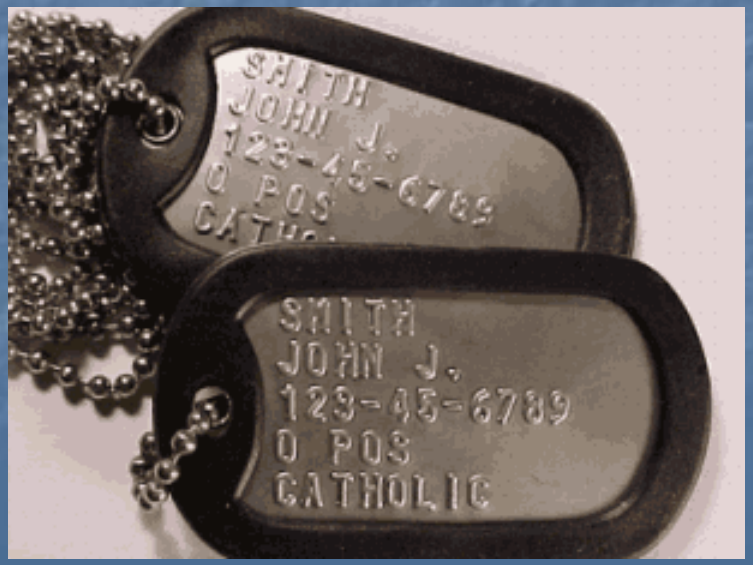
- DNA evidence-has many uses within the legal system and criminal cases.
  - Proving someone guilty or innocent for a crime they have or have not committed.
  - Identification
  - Paternity Testing

Criminal Cases

- DNA evidence has exonerated people accused of committing crimes.
- Only about 30% of all DNA tests run by the FBI have exonerated an accused person; DNA evidence is still not as useful as fingerprinting.

Identification

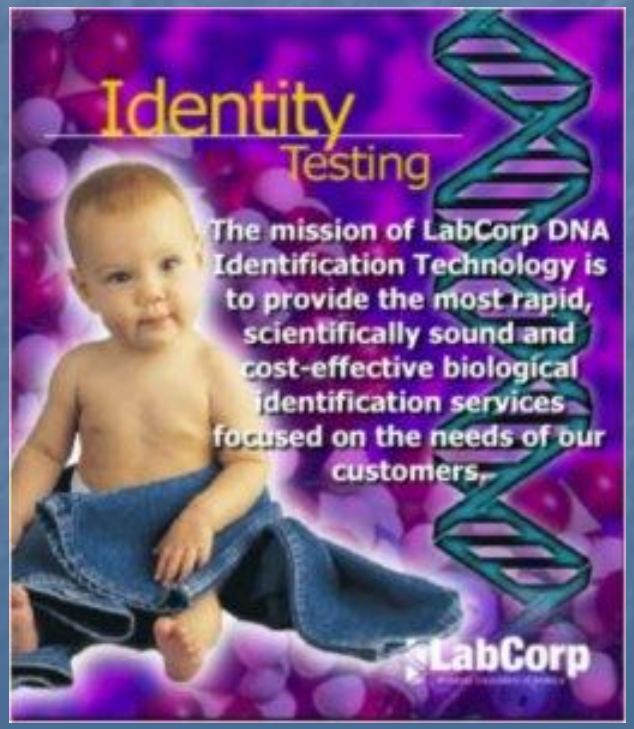
- Used to determine the sex, race, or even name of unnamed victims of crimes.
- Used in military to identify those who have died in battle, similar to the purpose of dog tags.

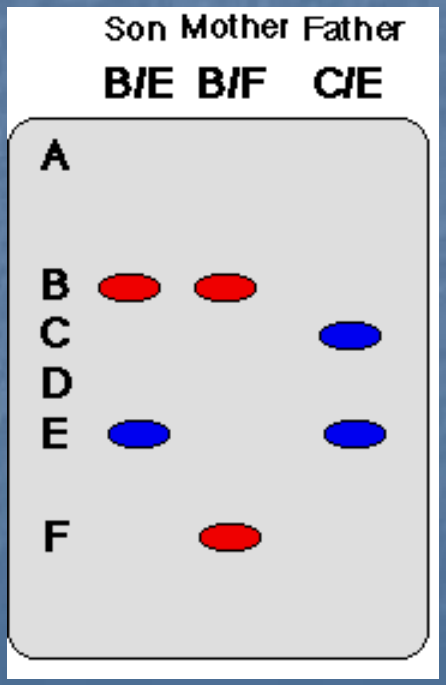


Typical dog tags

Paternity Testing

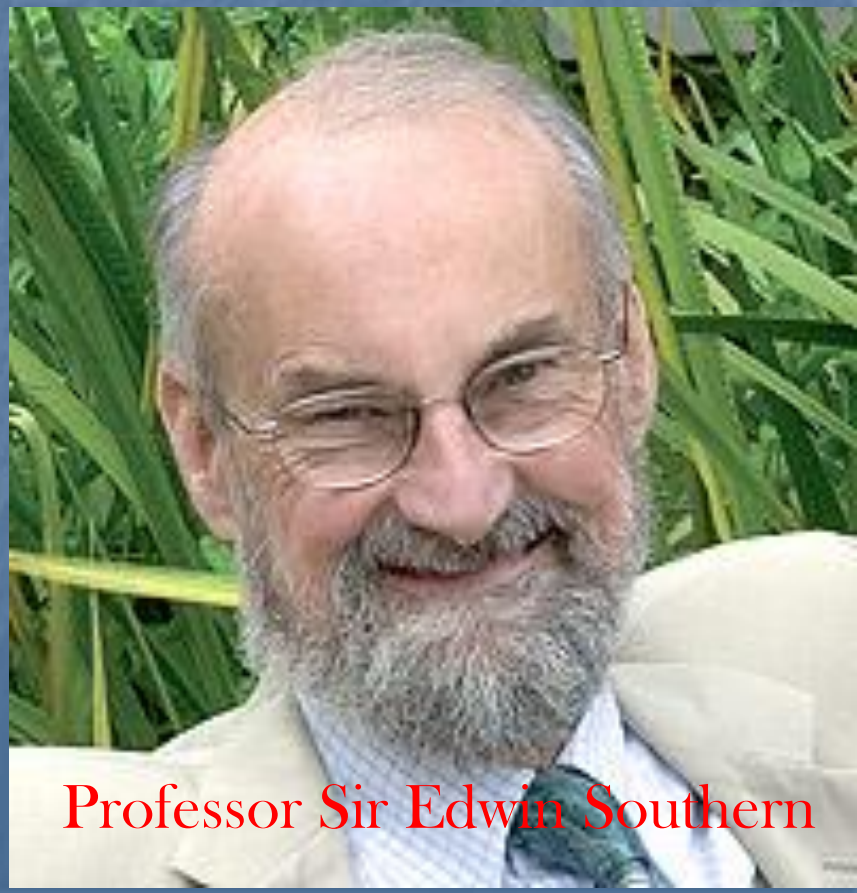
- Evidence can be used to compare the DNA of the suspected parent(s) and that of the child and determine the real parent.





SOUTHERN BLOTTING

- Professor Sir Edwin Southern, Professor of Biochemistry and Fellow of Trinity developed this method in 1975.
- Southern won the Lasker Award for Clinical Medical Research prize for the method of finding specific DNA sequences he developed this procedure at Edinburgh University more than 30 years ago. The technique is known as DNA transfer or 'Southern blotting'



Professor Sir Edwin Southern

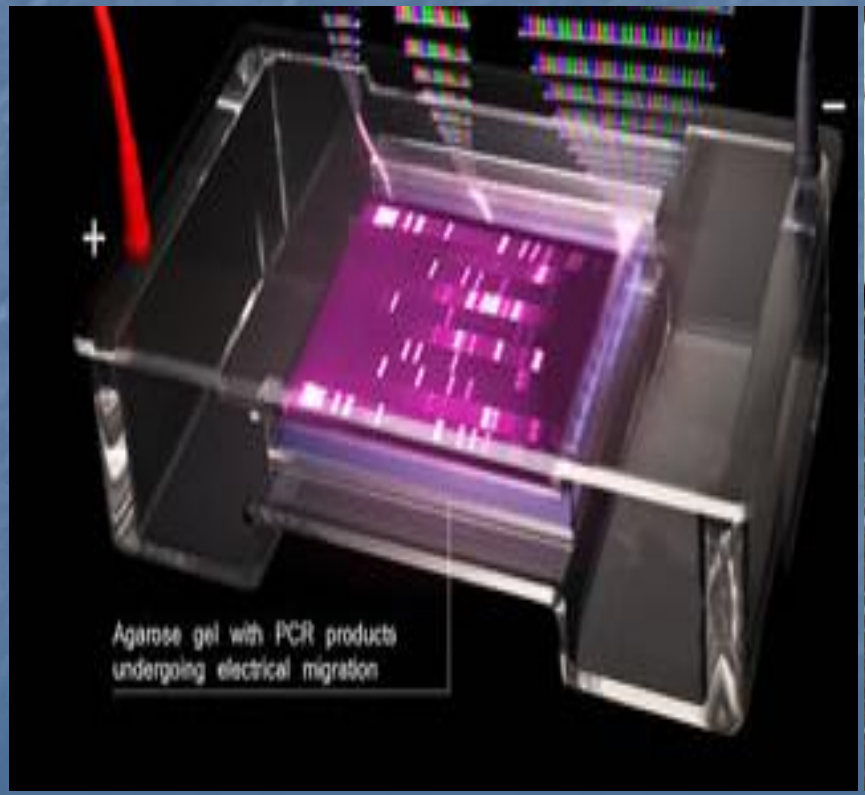
- This method Involves separation, transfer and hybridization.
- It is a method routinely used in molecular biology for detection of a specific DNA sequence in DNA samples.
- The DNA detected can be a single gene, or it can be part of a larger piece of DNA such as a viral genome.

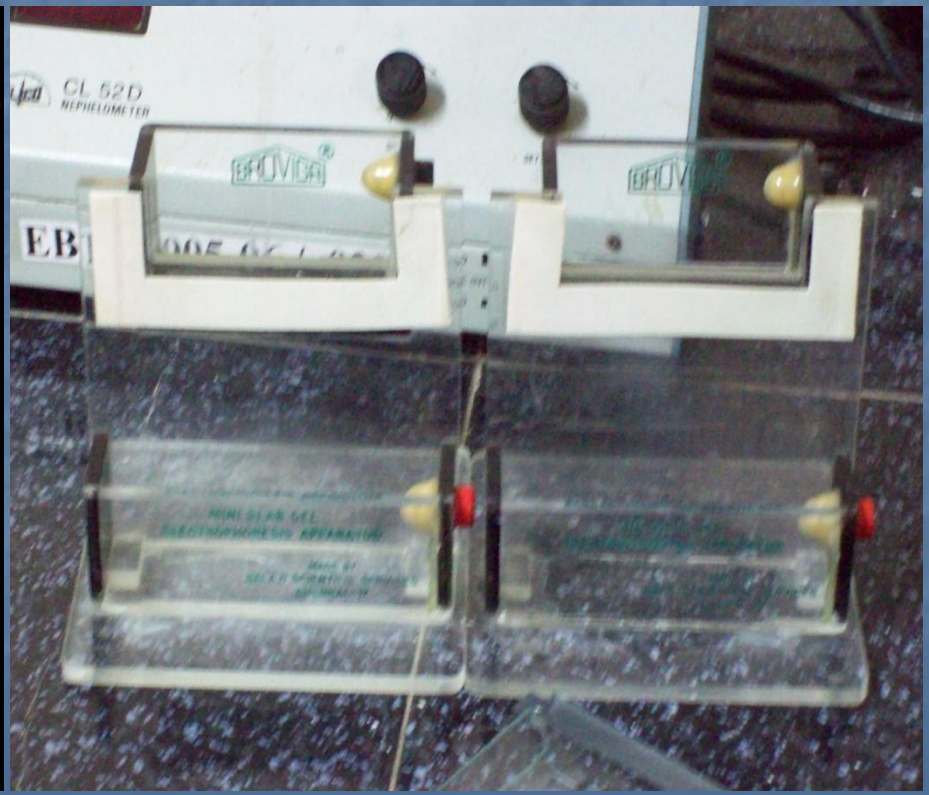
- Southern blotting combines agarose gel electrophoresis for size separation of DNA with methods to transfer the size separated DNA to a filter membrane for probe hybridization.
- The key to this method is Hybridization.
- Hybridization - Process of forming a double-stranded DNA molecule between a single-stranded DNA probe and a single-stranded target patient DNA.

PRINCIPLE

1. The mixture of molecules is separated.
2. The molecules are immobilized on a matrix.
3. The probe is added to the matrix to bind t molecules.
4. Any unbound probes are then removed.
5. The place where the probe is connected corresponds to the location of the immobilized target molecule.

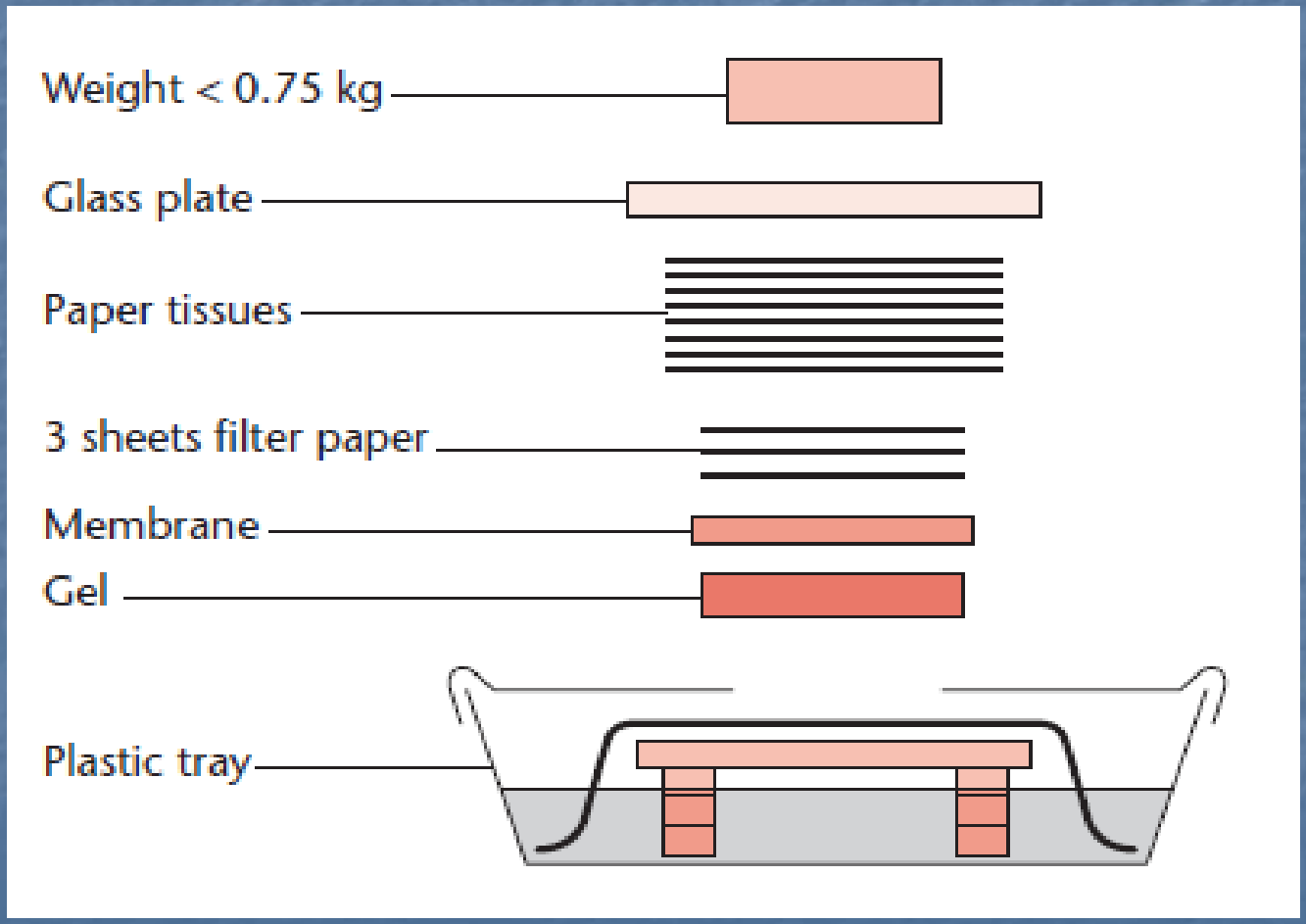
APPARATUS


+
-
Agarose gel with PCR products
undergoing electrical migration


CL 52D
NEPHELOMETER
BIOVICA
BIOVICA

Whatman 3MM paper

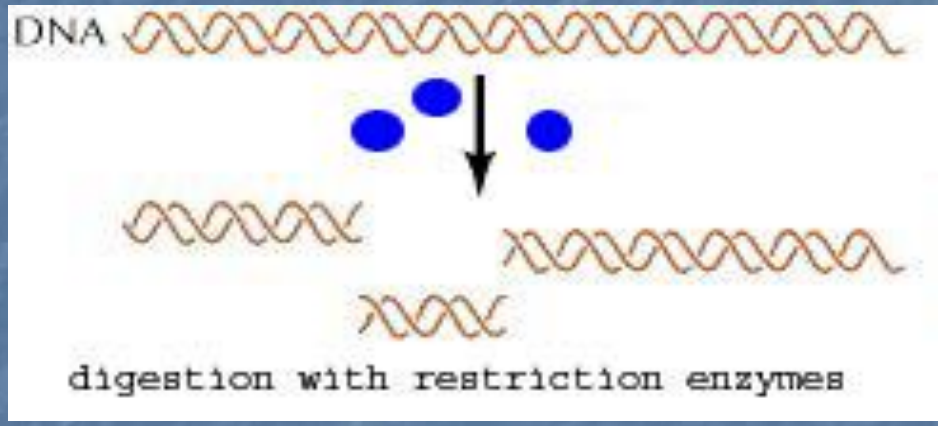
nitrocellulosemembrane



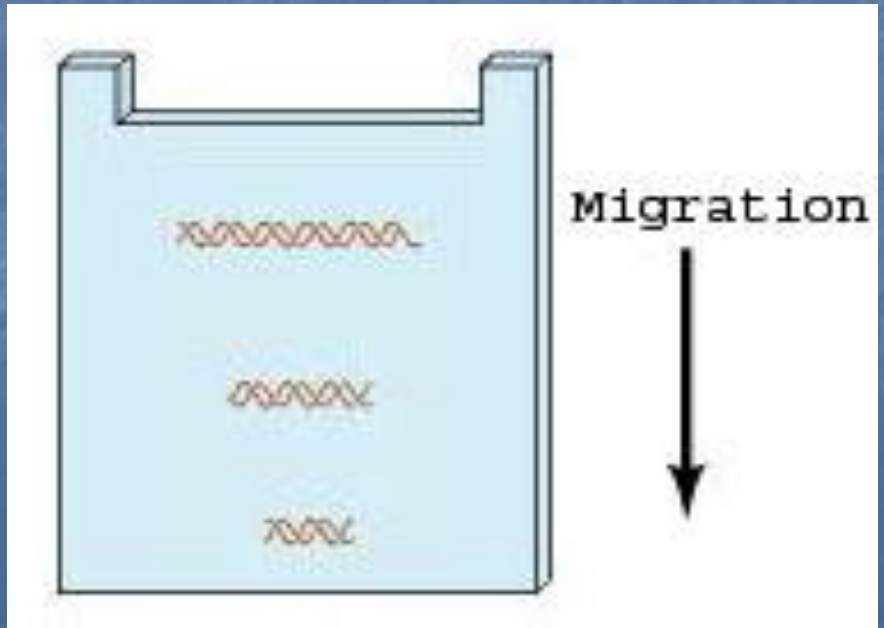
Capillary plotting apparatus

Steps in southern blotting

1. Digest the DNA with an appropriate restriction enzyme.

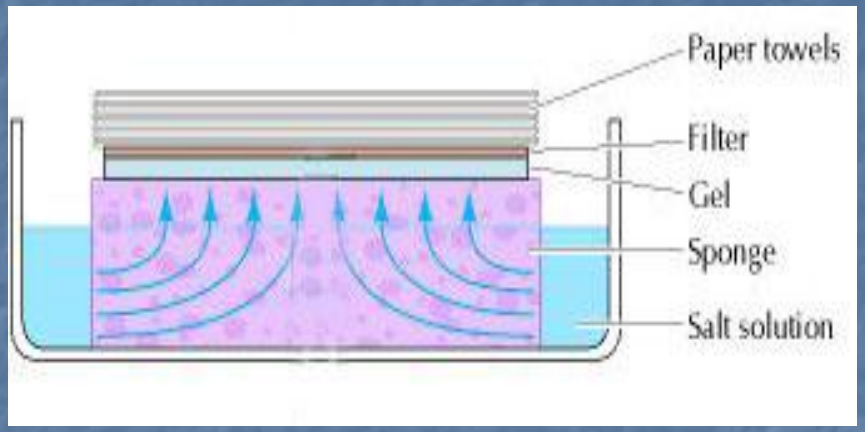


2. The complex mixture of fragments is subjected to gel electrophoresis to separate the fragments according to size.

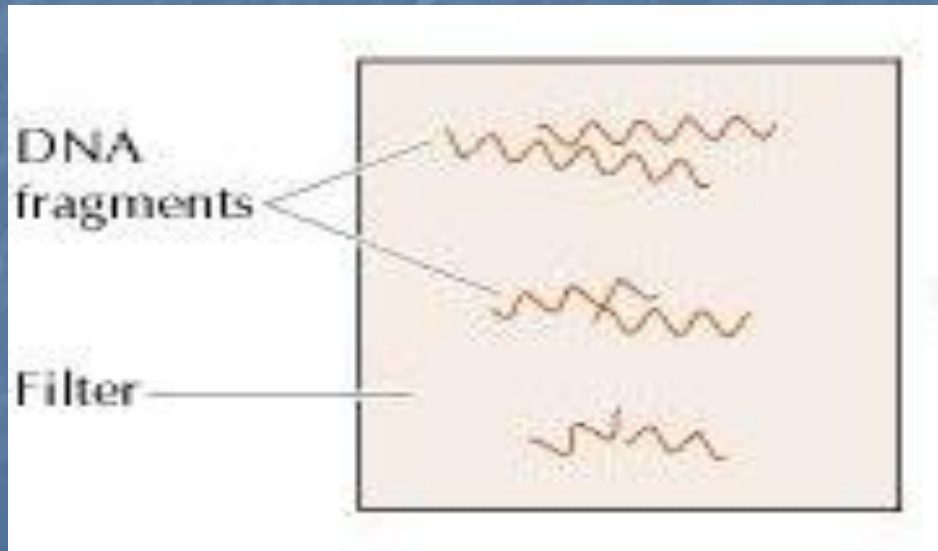


3.The restriction fragments present in the gel are denatured with alkali and transferred onto

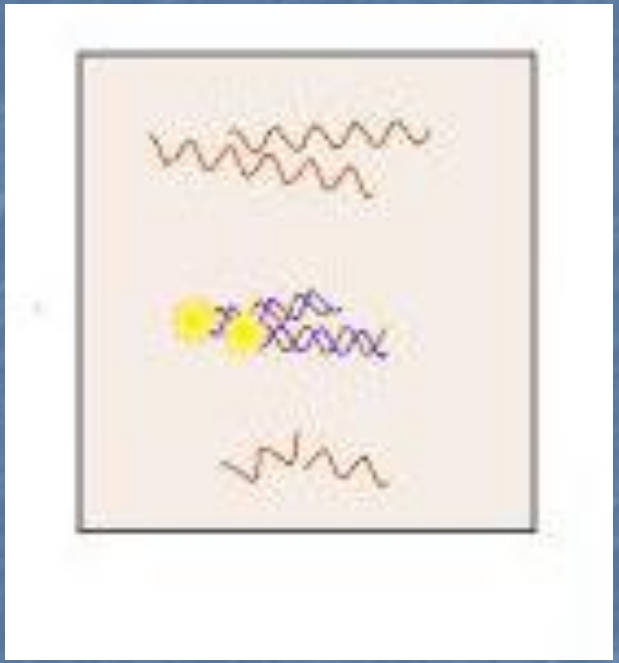
4. a nitrocellulose filter or nylon membrane by blotting.



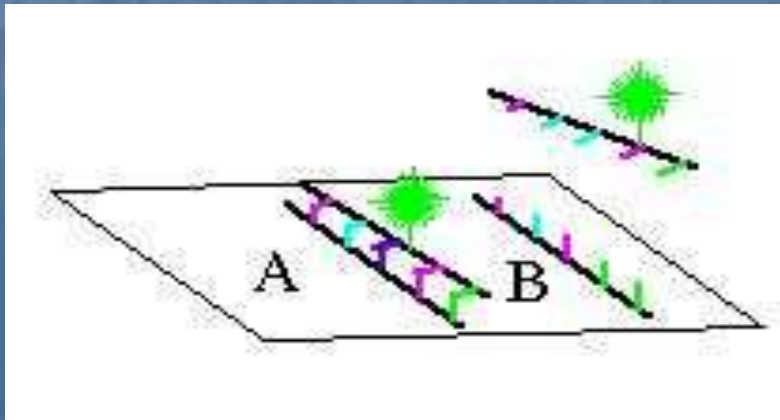
- This procedure preserves the distribution of the fragments in the gel, creating a replica of the gel on the filter.



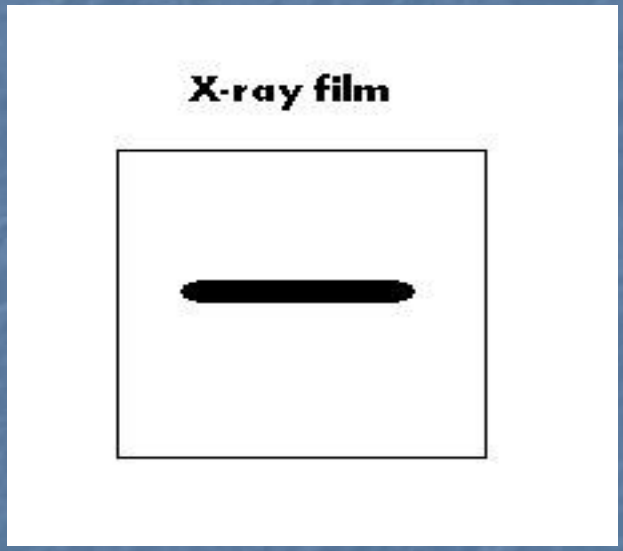
5.The filter is incubated under hybridization conditions with a specific radiolabeled DNA probe.

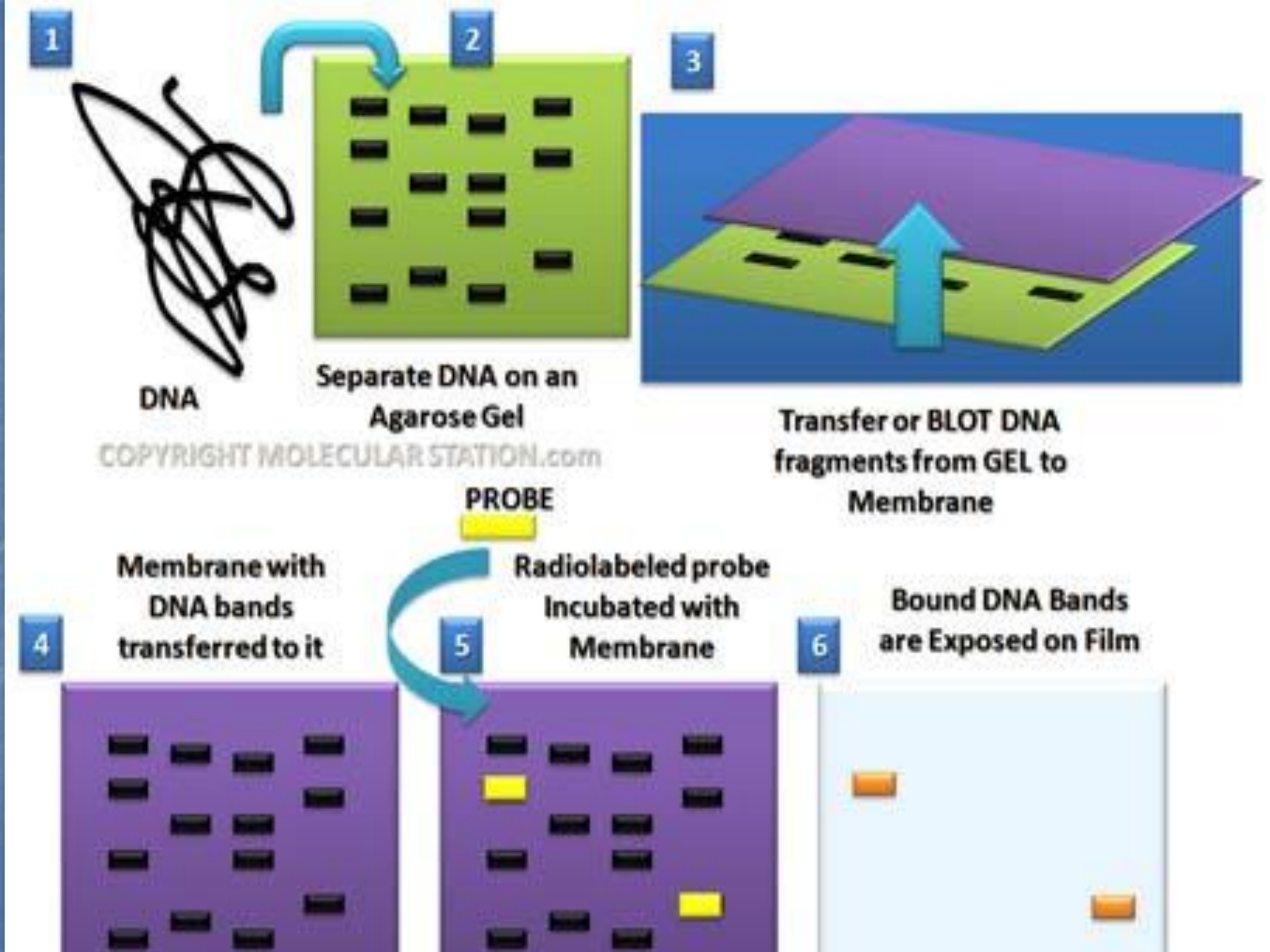


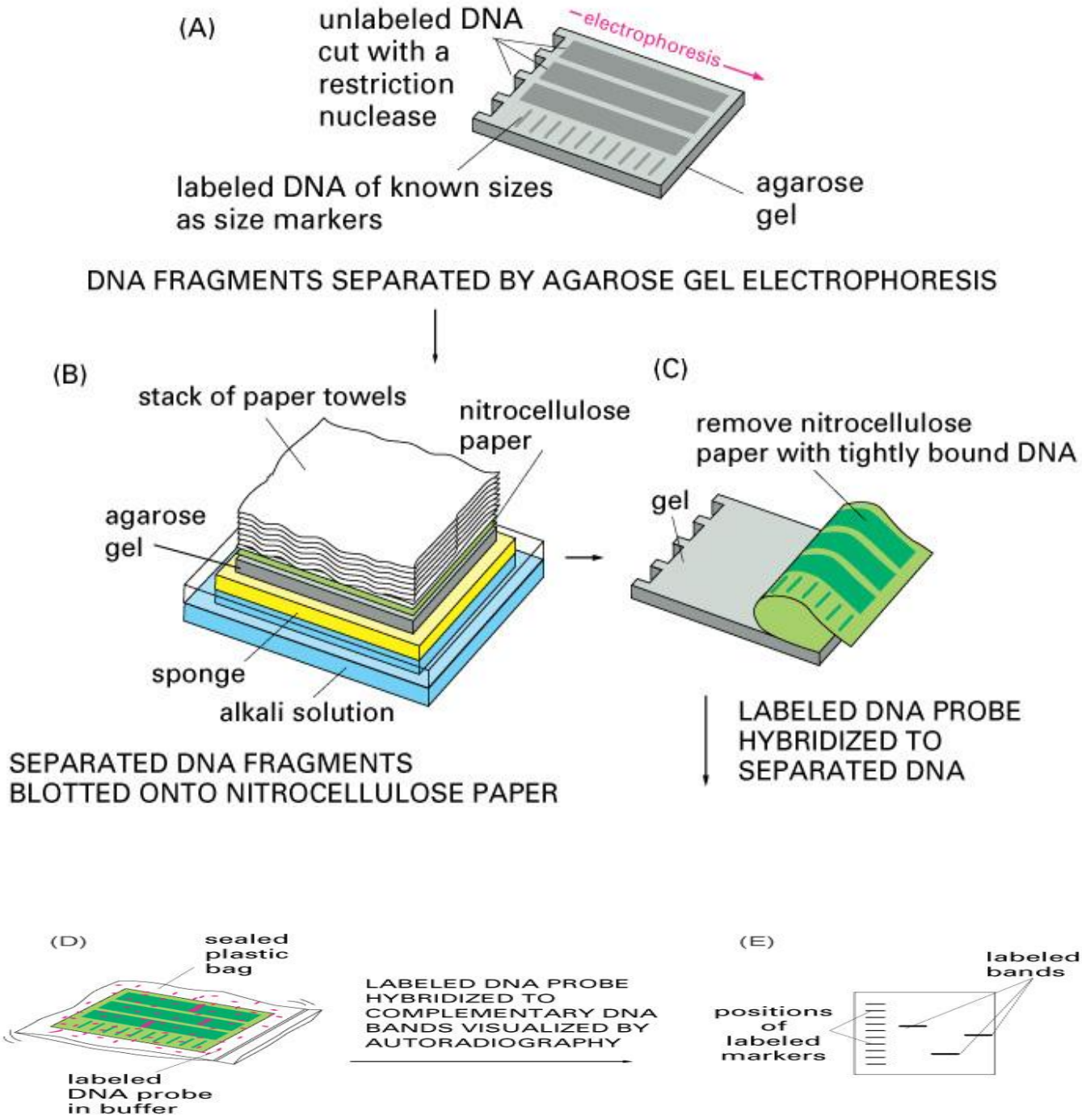
- The probe hybridizes to the complementary DNA restriction fragment.



6.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.




1
2
3
DNA
Separate DNA on an Agarose Gel
Transfer or BLOT DNA fragments from GEL to Membrane
COPYRIGHT MOLECULAR STATION.com
PROBE
Membrane with DNA bands transferred to it
Radiolabeled probe Incubated with Membrane
Bound DNA Bands are Exposed on Film
4
5
6


(A)
unlabeled DNA cut with a restriction nuclease
electrophoresis
labeled DNA of known sizes as size markers
agarose gel
DNA FRAGMENTS SEPARATED BY AGAROSE GEL ELECTROPHORESIS
(B)
stack of paper towels
nitrocellulose paper
agarose gel
sponge
alkali solution
SEPARATED DNA FRAGMENTS BLOTTED ONTO NITROCELLULOSE PAPER
(C)
remove nitrocellulose paper with tightly bound DNA
gel
LABELED DNA PROBE HYBRIDIZED TO SEPARATED DNA
(D)
sealed plastic bag
labeled DNA probe in buffer
LABELED DNA PROBE HYBRIDIZED TO COMPLEMENTARY DNA BANDS VISUALIZED BY AUTORADIOGRAPHY
(E)
labeled bands
positions of labeled markers

APPLICATIONS

- Southern blots are used in gene discovery , mapping, evolution and development studies, diagnostics and forensics (It is used for DNA fingerprinting, preparation of RFLP maps)
- identification of the transferred genes in transgenic individuals, etc.

APPLICATIONS

- Southern blots allow investigators to determine the molecular weight of a restriction fragment and to measure relative amounts in different samples.
- Southern blot is used to detect the presence of a particular bit of DNA in a sample
- analyze the genetic patterns which appear in a person's DNA.

NORTHERN BLOTTING

- The northern blot is used to study the expression patterns of a specific type of RNA molecule as relative comparison among a set of different samples of RNA.

- RNA is separated based on size and is then transferred to a membrane then probed with a labeled complement of a sequence of interest.

- The results may be visualized through a variety of ways depending on the label used. Most result in the revelation of bands representing the sizes of the RNA detected in sample.

- The intensity of these bands is related to the amount of the target RNA in the samples analyzed.

- It is used to study when and how much gene expression is occurring by measuring how much of that RNA is present in different samples.

- one of the most basic tools for determining at what time, and under what conditions, certain genes are expressed in living tissues.

WESTERN BLOTTING

- In western blotting, proteins are first separated by size, in a thin gel sandwiched between two glass plates in a technique known as SDS-PAGE sodium dodecyl sulphate polyacrylamide gel electrophoresis.

- The proteins in the gel are then transferred to a nitrocellulose, nylon or other support membrane.

- This membrane probed with solutions of antibodies. Antibodies specifically bind to the protein of interest & visualized by a variety of techniques, including colored products, chemiluminescence, or autoradiography.

- Antibodies are labeled with enzymes. When a chemiluminescent substrate is exposed to the enzyme it allows detection.

- Using western blotting techniques allows not only detection but also quantitative analysis.

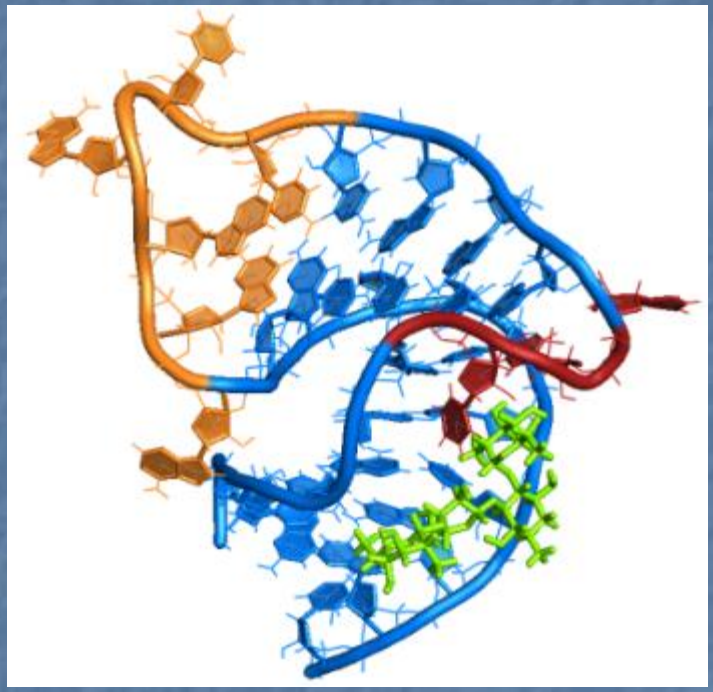
Northern Blotting

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

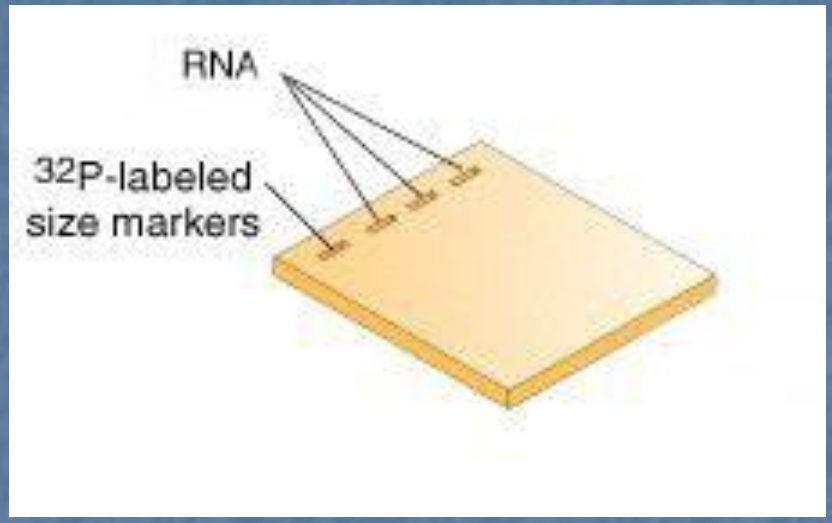
Steps involved in Northern blotting

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

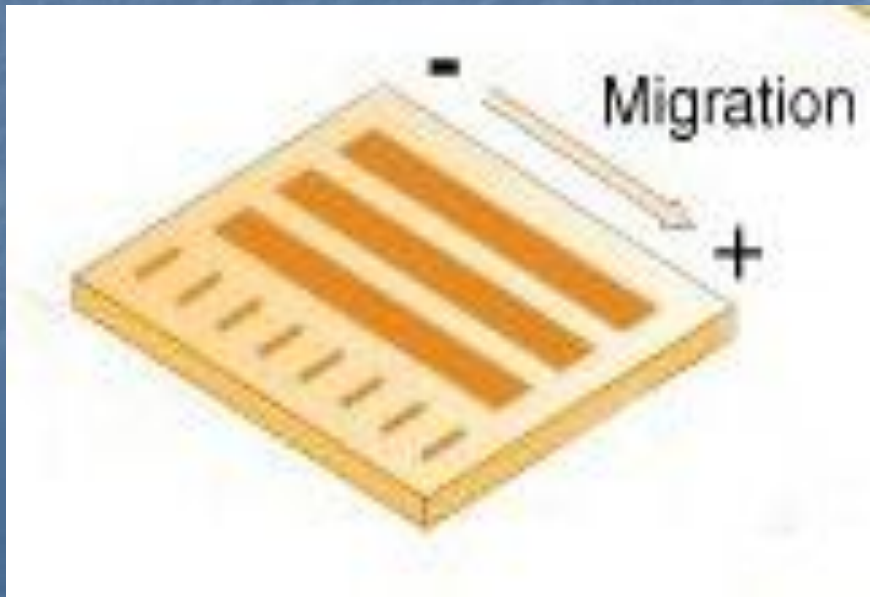
   * RNA is more susceptible to degradation than DNA.



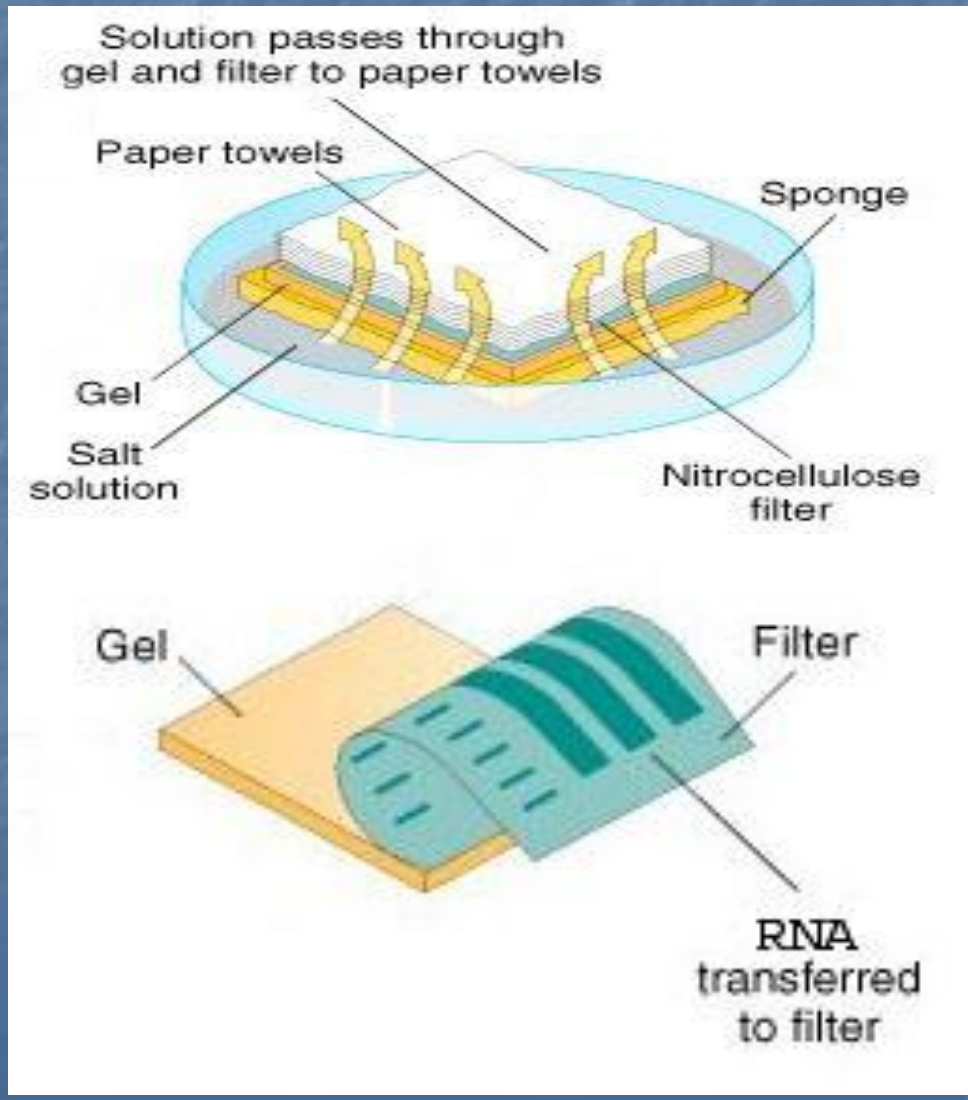
2. SAMPLE'S ARE LOADED ON GEL AND THE RNA SAMPLES ARE SEPARATED ACCORDING TO THEIR SIZE ON AN AGAROSE GEL .



- THE RESULTING GEL FOLLOWING AFTER THE ELECTROPHORESIS RUN.



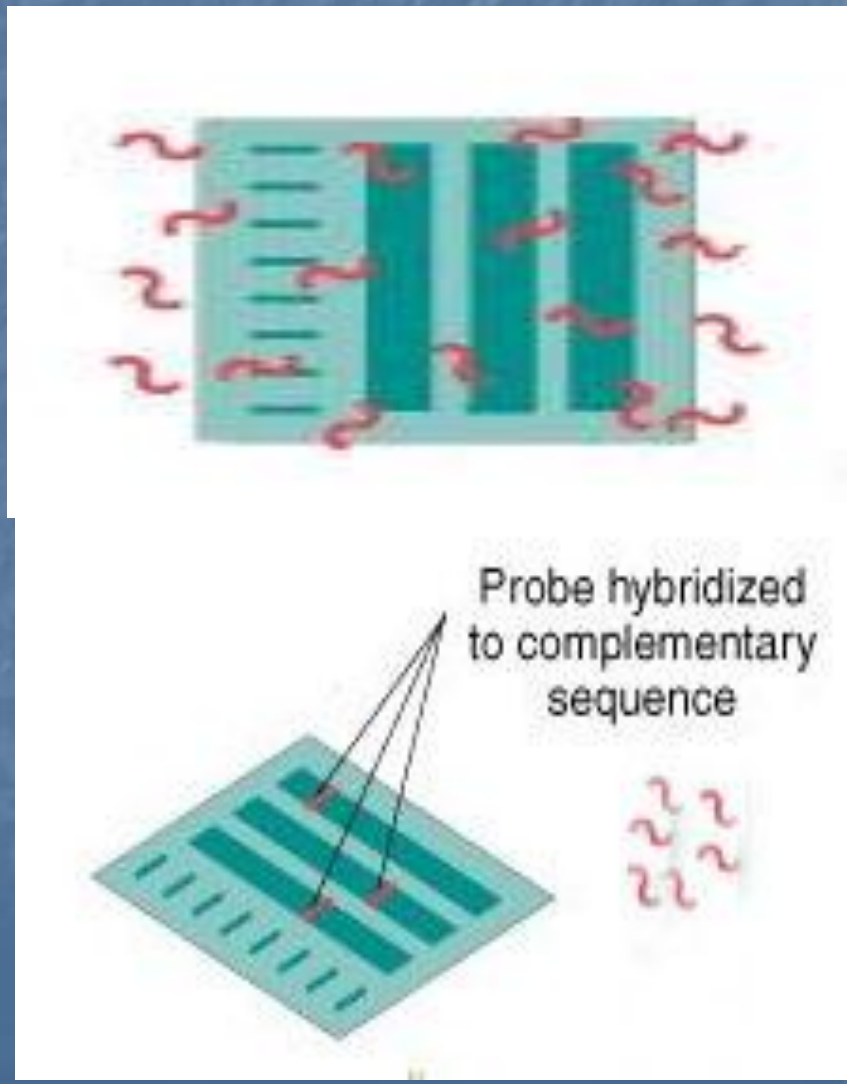
3. THE GEL IS THEN BLOTTED ON A NYLON MEMBRANE OR A NITROCELLULOSE FILTER PAPER BY CREATING THE SANDWICH ARRANGEMENT.



4. THE MEMBRANE IS PLACED IN A DISH CONTAINING HYBRIDIZATION BUFFER WITH A LABELED PROBE.

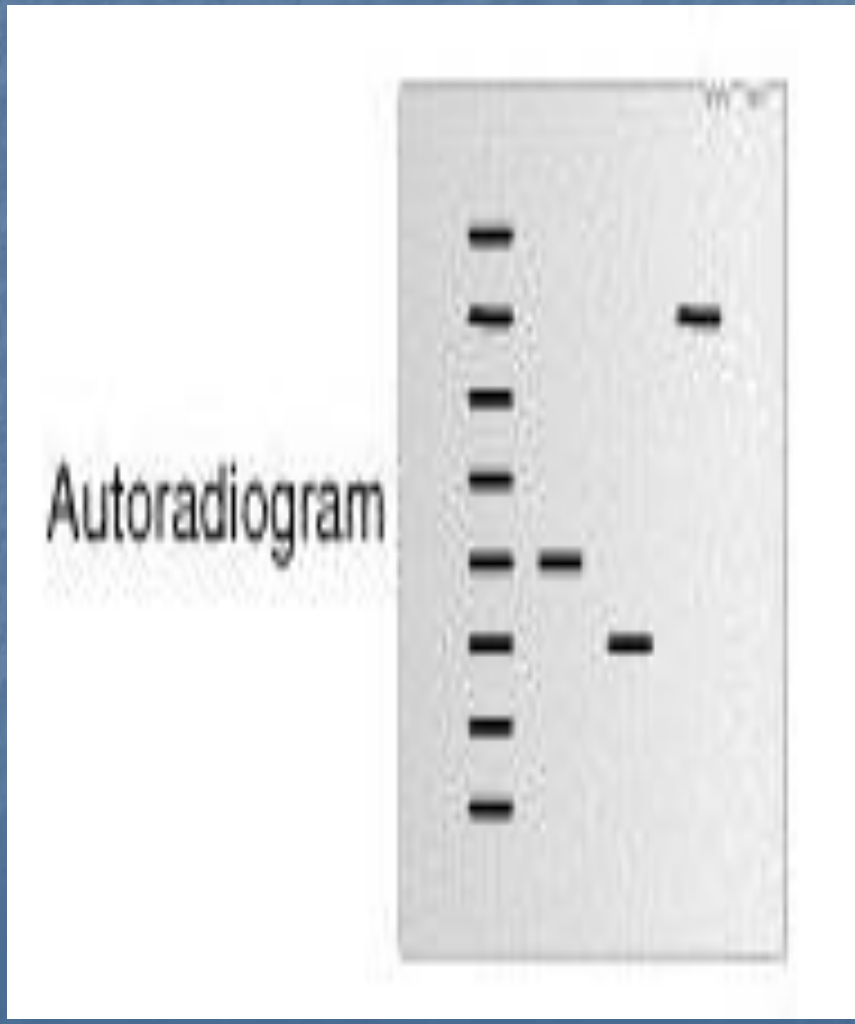
- THUS, IT WILL HYBRIDIZE TO THE RNA ON THE BLOT THAT CORRESPONDS TO THE SEQUENCE OF INTEREST.

5. THE MEMBRANE IS WASHED TO REMOVE UNBOUND PROBE.



6. 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.

- NOW THE EXPRESSION PATTERNS OF THE SEQUENCE OF INTEREST IN THE DIFFERENT SAMPLES CAN BE COMPARED.



APPLICATIONS

- A STANDARD FOR THE STUDY OF GENE EXPRESSION AT THE LEVEL OF MRNA (MESSENGER RNA TRANSCRIPTS)
- DETECTION OF MRNA TRANSCRIPT SIZE
- STUDY RNA DEGRADATION
- STUDY RNA SPLICING
- STUDY RNA HALF-LIFE
- OFTEN USED TO CONFIRM AND CHECK TRANSGENIC / KNOCKOUT MICE (ANIMALS)

Disadvantage of Northern plotting

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.

Western blotting

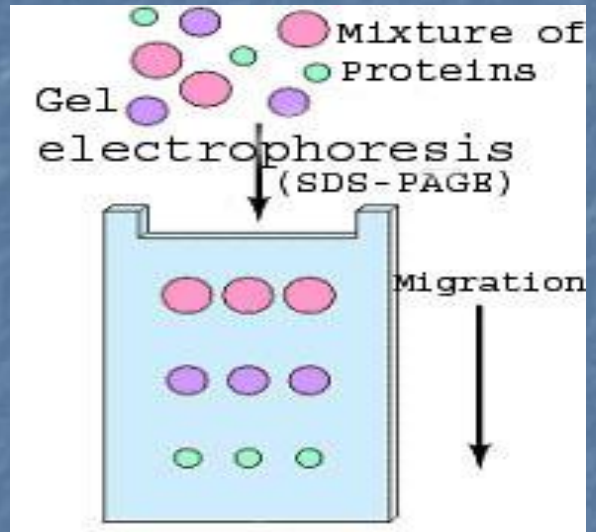
- Western blotting (1981) is an immunoblotting technique which rely on the specificity of binding between a protein of interest and a probe (antibody raised against that particular protein) to allow detection of the protein of interest in a mixture of many other similar molecules.

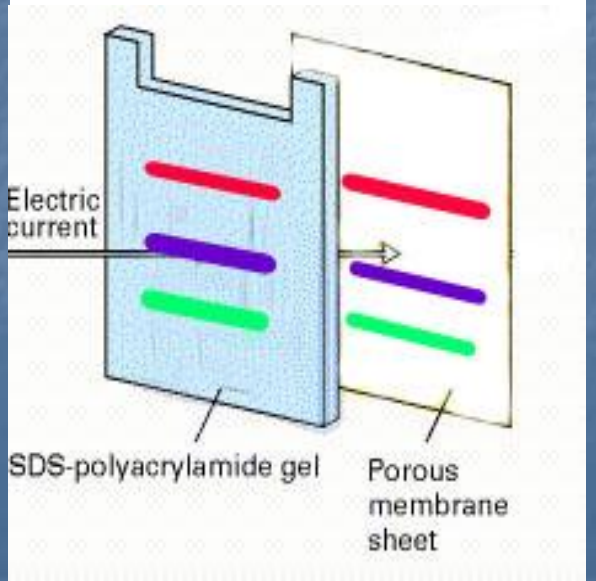
- The SDS page technique is a prerequisite for western blotting .

Steps in western blotting

1. A protein sample is subjected to electrophoresis on an SDS-polyacrylamide gel.

2. Electroblotting transfers the separated proteins from the gel to the

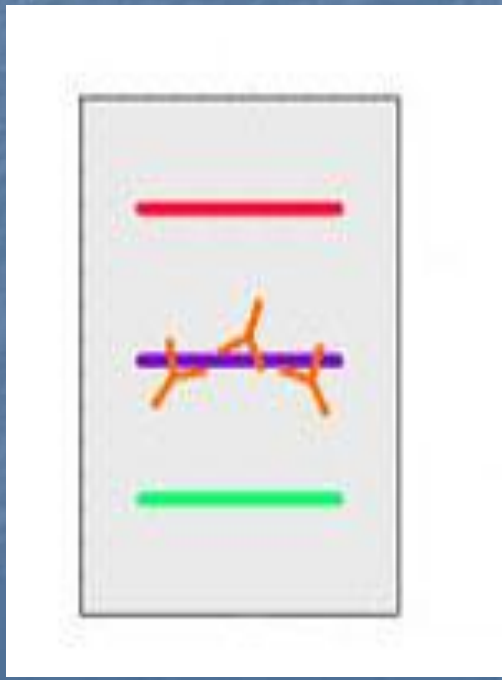




Cont…

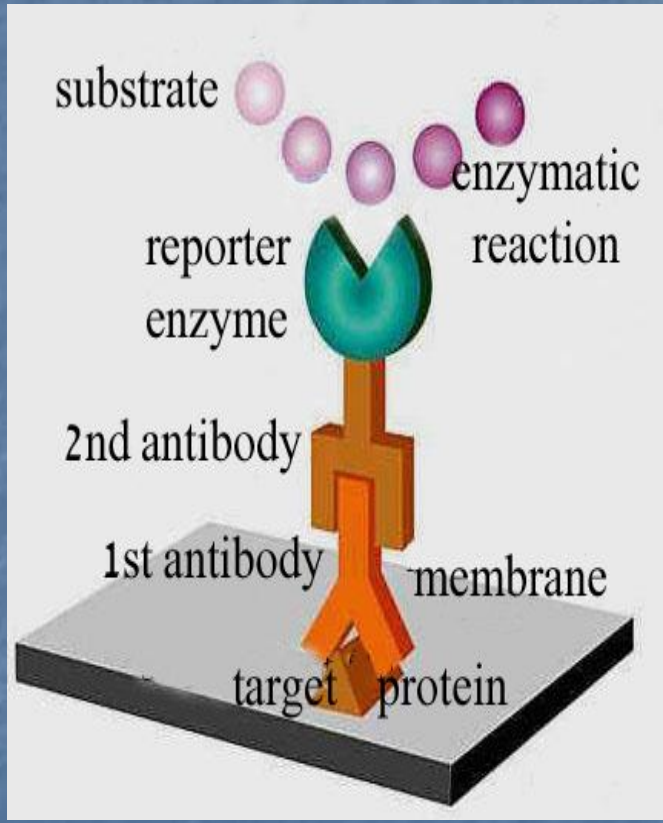
3. The blot is incubated with a generic protein (such as milk proteins or BSA) which binds to any remaining sticky places on the nitrocellulose.

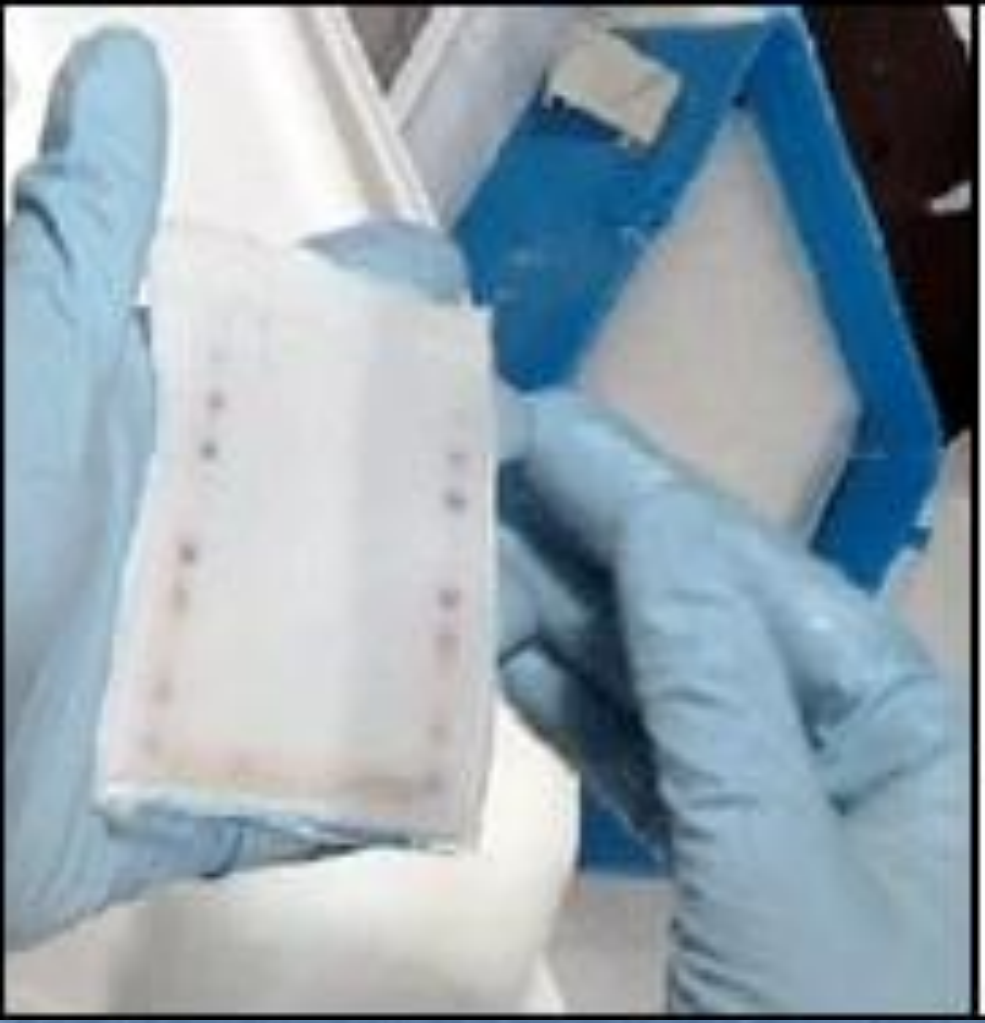
4. An antibody that is specific for the protein of interest (the primary antibody – Ab1) is added to the nitrocellulose sheet and reacts with the antigen. Only the band

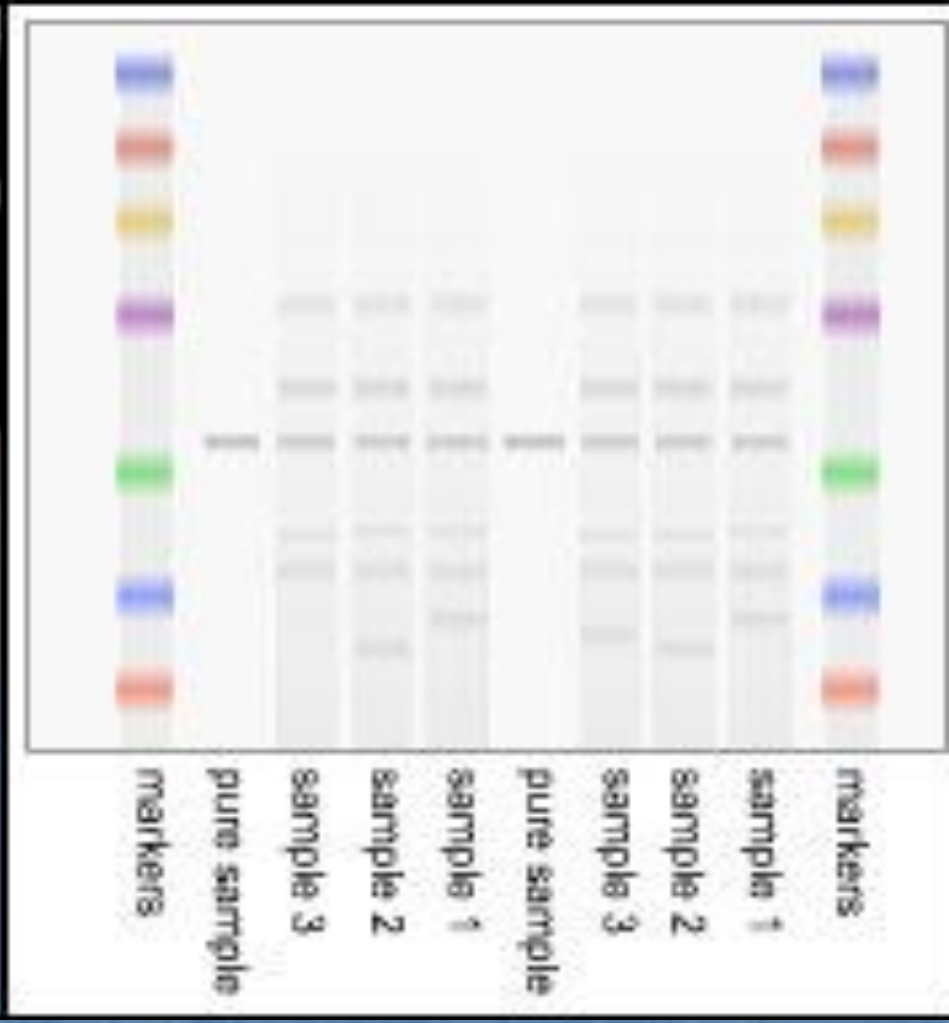


Cont…

5. After washing for removal of non-specifically bound AB1, second antibody (AB2)is added, which specifically recognizes the Fc domain of the primary antibody and binds it. AB2 is radioactively labeled, or is covalently linked to a reporter enzyme, which allows to visualize the protein-AB1-AB2 complex





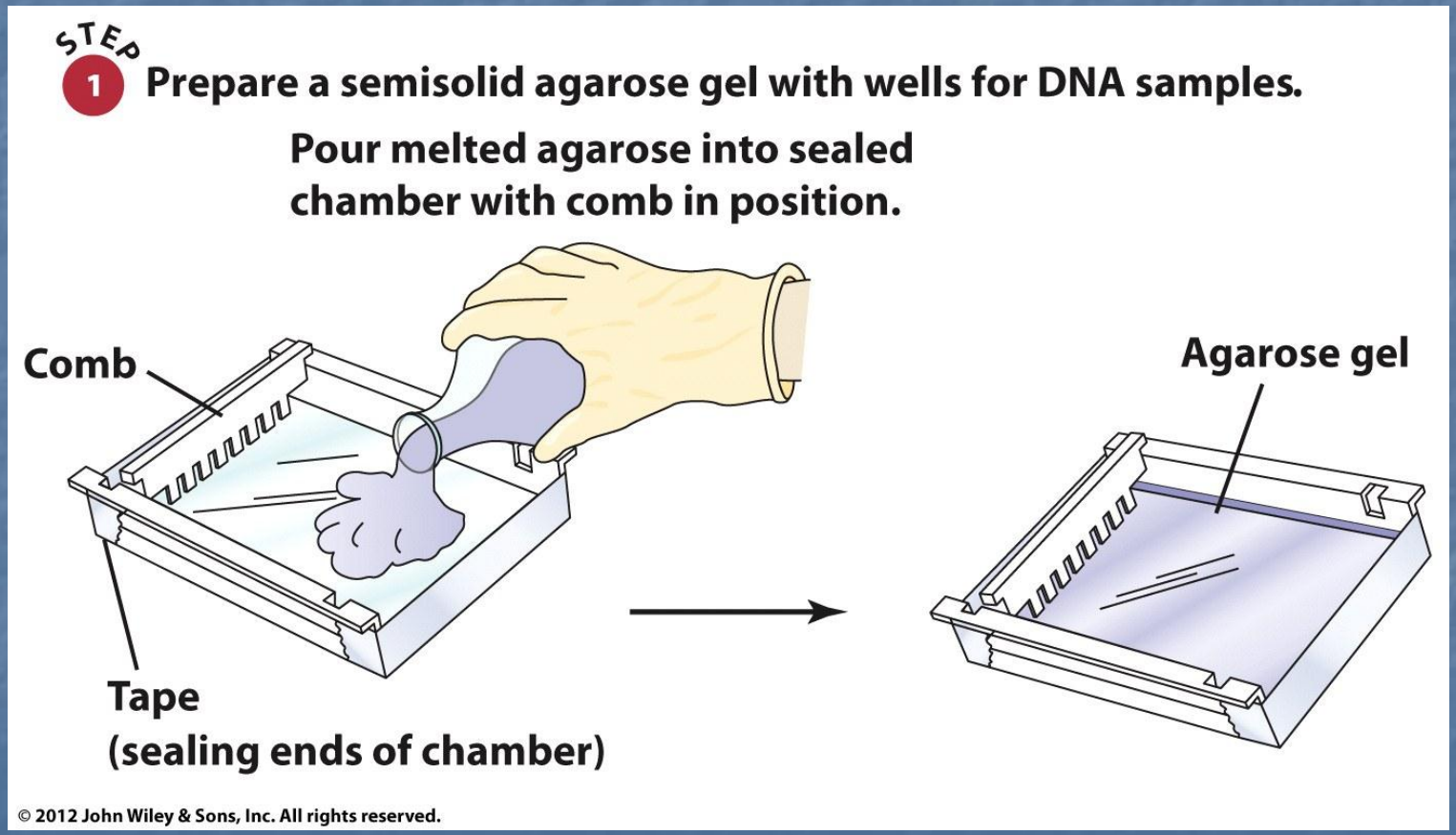

markers
sample 1
sample 2
sample 3
pure sample
sample 1
sample 2
sample 3
pure sample
markers

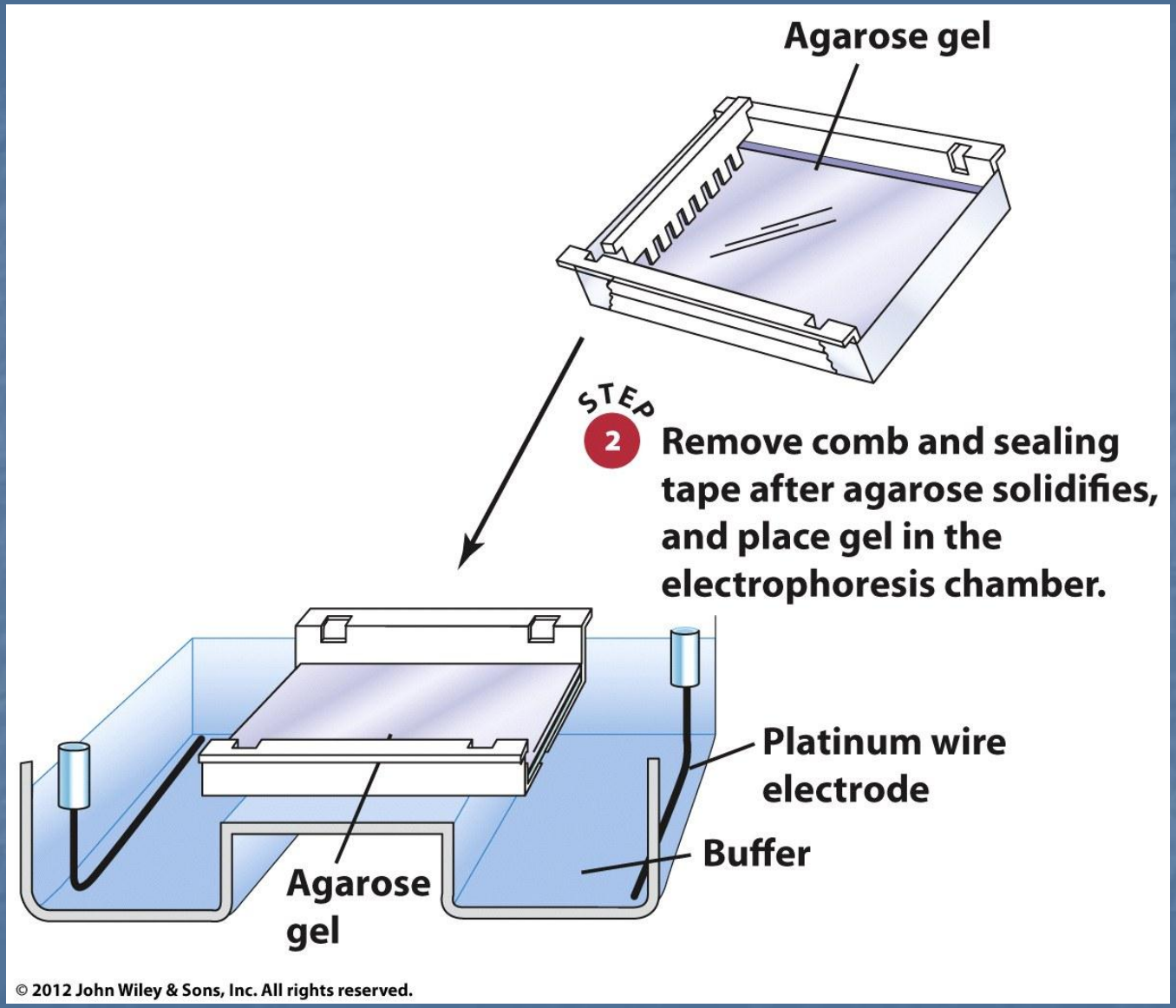
An example

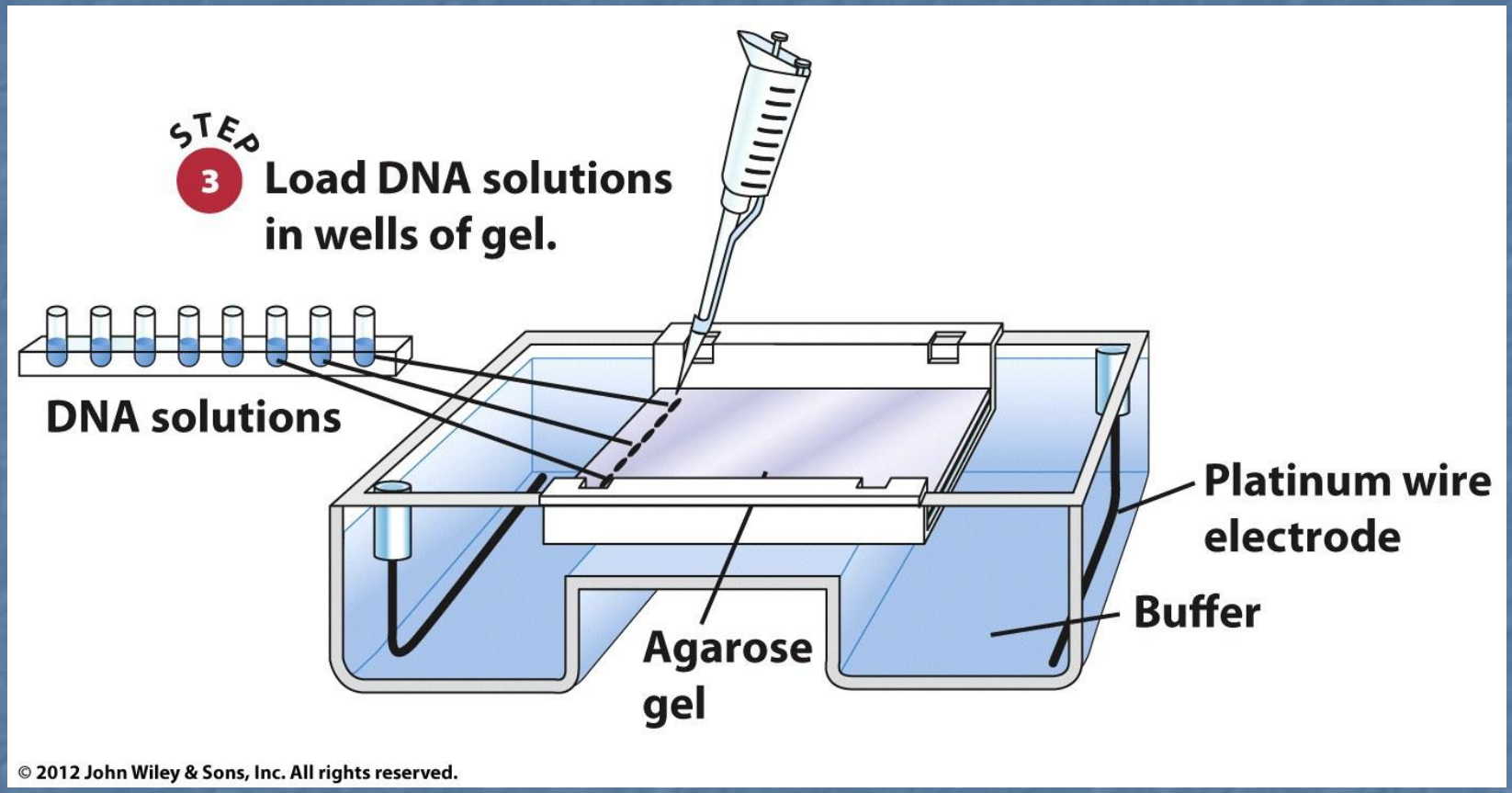
Application

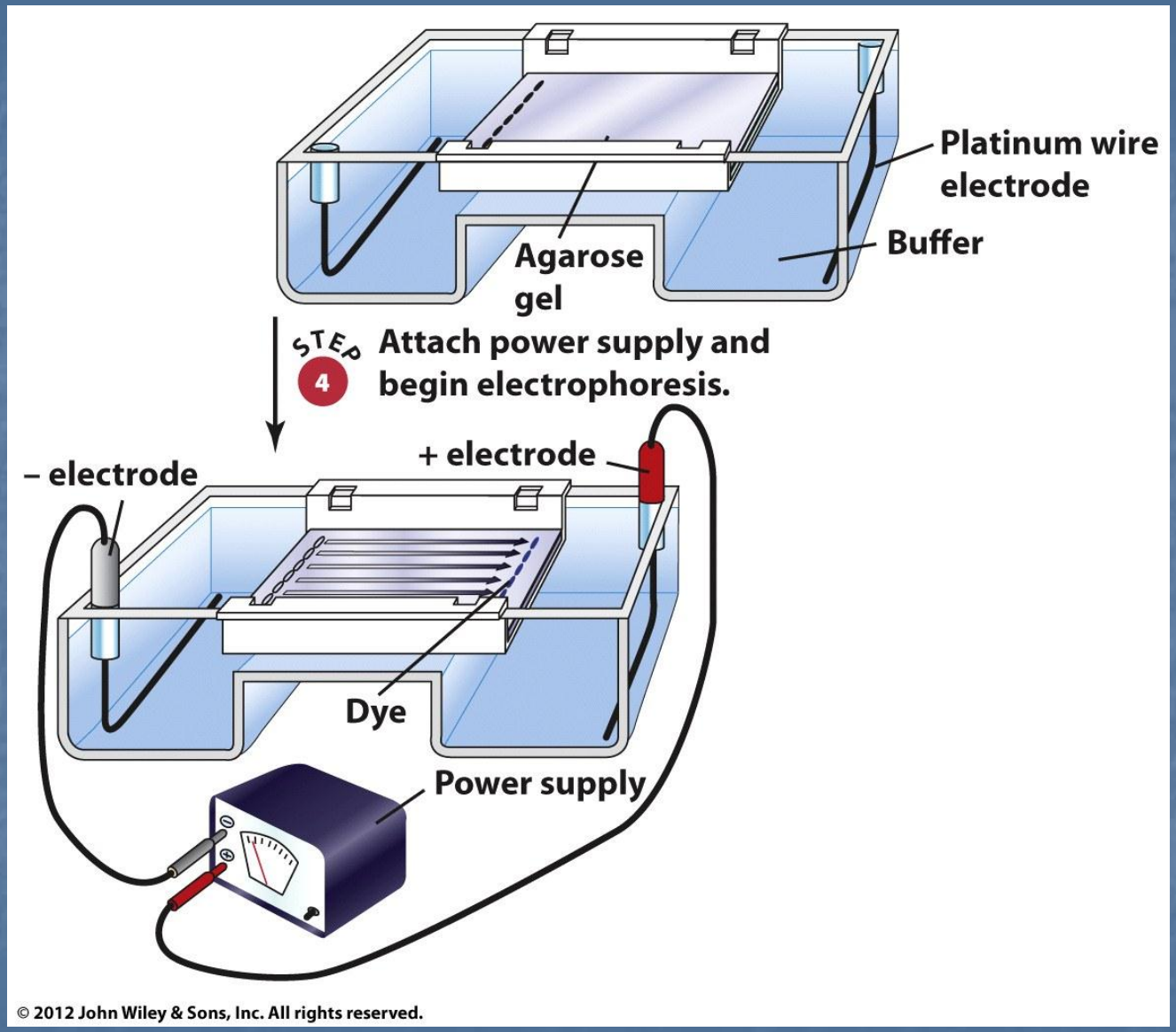
1. The confirmatory HIV test
2. Western blot is also used as the definitive test for Bovine spongiform encephalopathy (BSE)
3. Some forms of Lyme disease testing employ Western blotting.

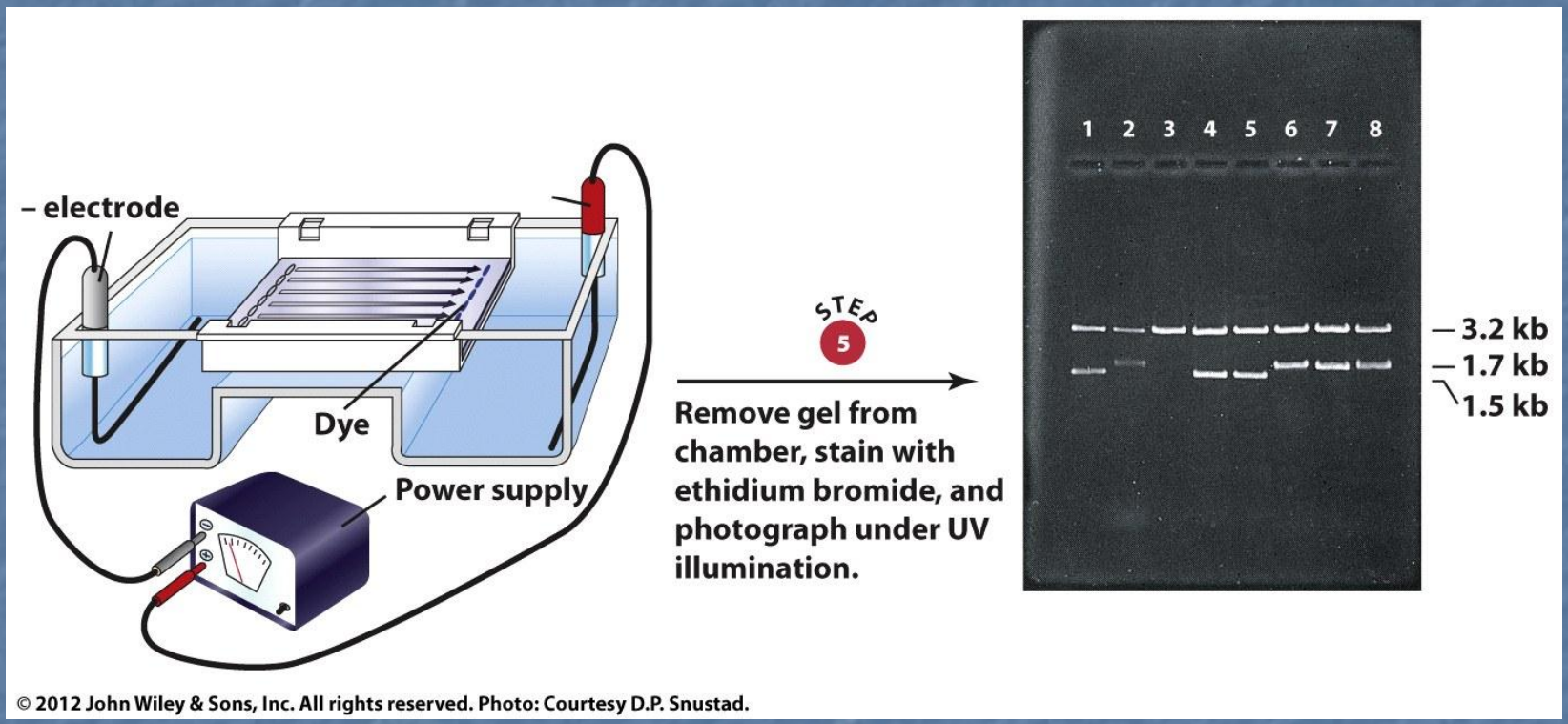
Agarose Gel Electrophoresis



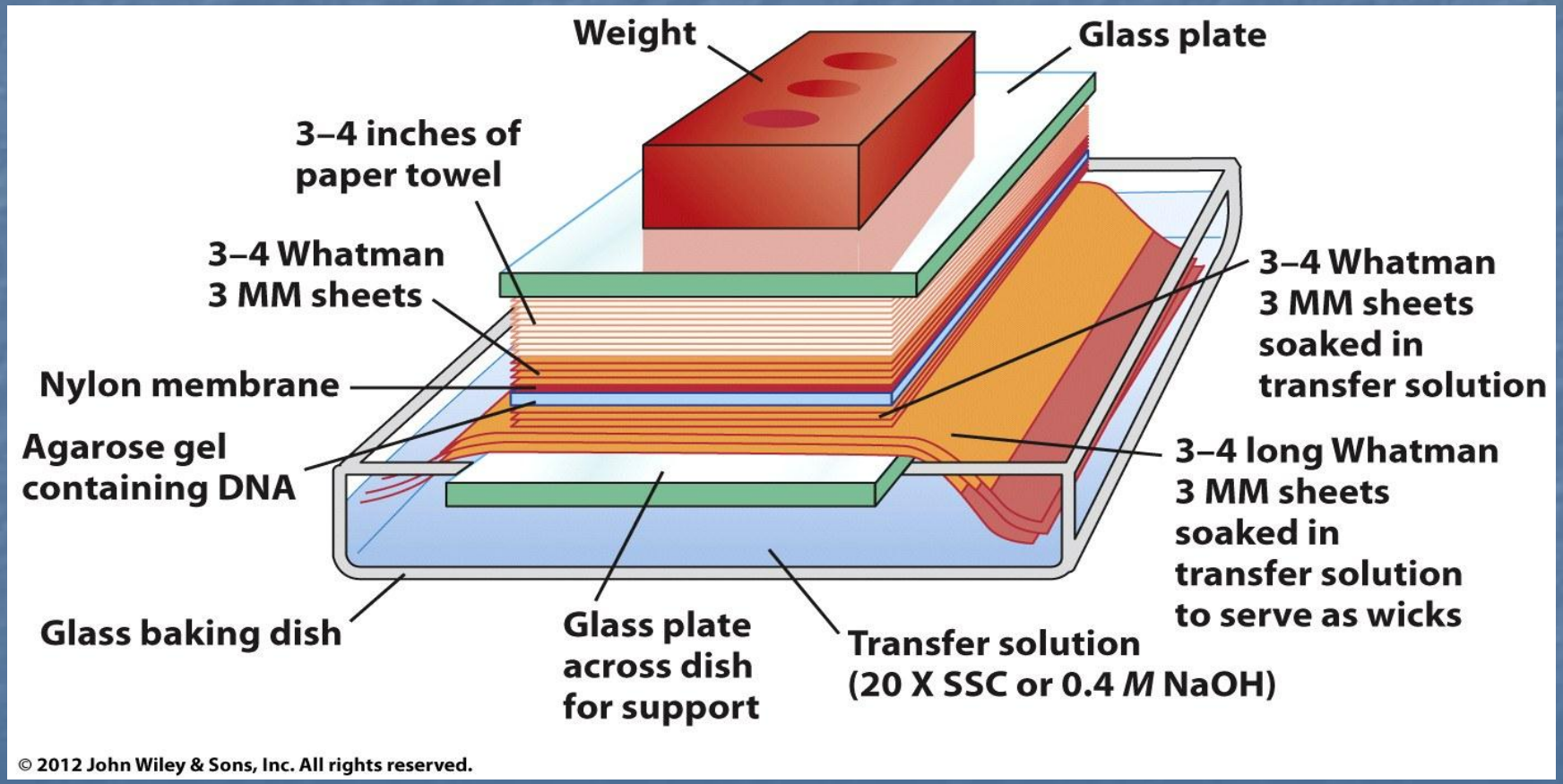








Southern Blot: Transferring DNA from the Gel to a Membrane



Identification of a Specific Fragment by Southern Blot Hybridization