RAMA UNIVERSITY

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FACULTY OF ENGINEERING &TECHNOLOGY

DEPARTMENT OF BIOTECHNOLOGY

SOUTHERN BLOTTING (HYBRIDIZATION)

Blotting techniques are used to transfer DNA or RNA fragments or proteins from electrophoresis gel to a nitrocellulose sheet or nylon membrane as blotting paper is used to blot ink.

Southern blotting isthe transfer of DNA fragments from an electrophoresis gel to a membranous support which results in immobilization of DNA fragments. These immobilized single stranded DNA fragments can then be subjected to hybridization with a labeled probe.

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.

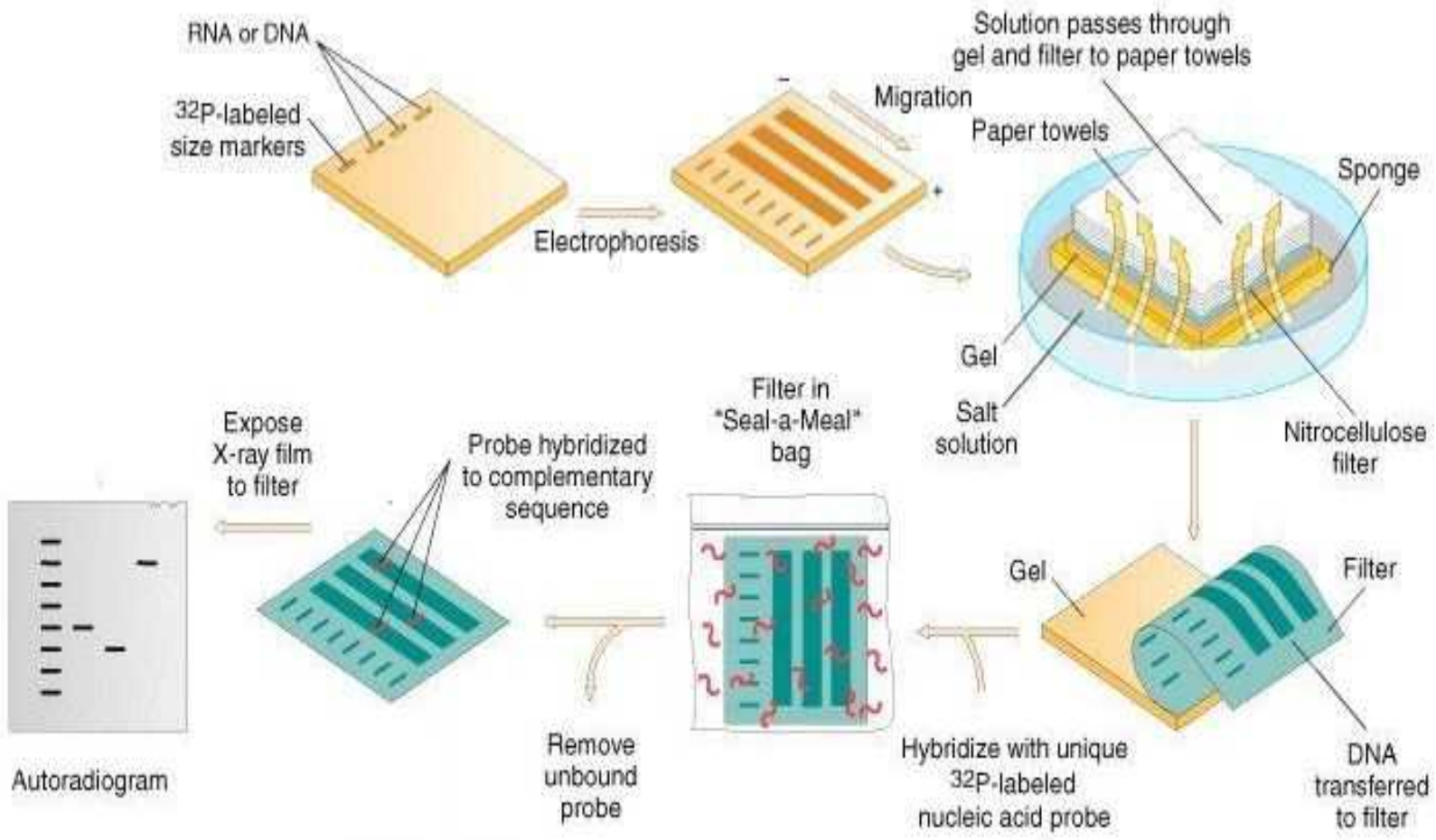
6 The filter is then exposed to X-ray film. The fragment containing the gene of interest is identified by a band on the developed film.

Southern blotting was named after Edward M. Southern who developed this procedure at Edinburgh University in the 1975.

It allows investigators to locate a particular sequence of DNA within a complex mixture.

DNA (genomic or other source) is digested with a restriction enzyme and separated by gel electrophoresis and transferred from an agarose gel onto a Nitrocellulose sheet or Nylon membrane which is then incubated with a single stranded DNA probe with known sequence. This probe is supposed to form base pairs with its complementary DNA sequence and to form a double-stranded DNA molecule.

The probe is labeled before hybridization either radioactively or is treated enzymatically by alkaline phosphotase or horseradish peroxidase.


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
Gel
Filter
DNA transferred to filter
Hybridize with unique 32P-labeled nucleic acid probe
Remove unbound probe
Filter in "Seal-a-Meal" bag
Probe hybridized to complementary sequence
Expose X-ray film to filter
Autoradiogram

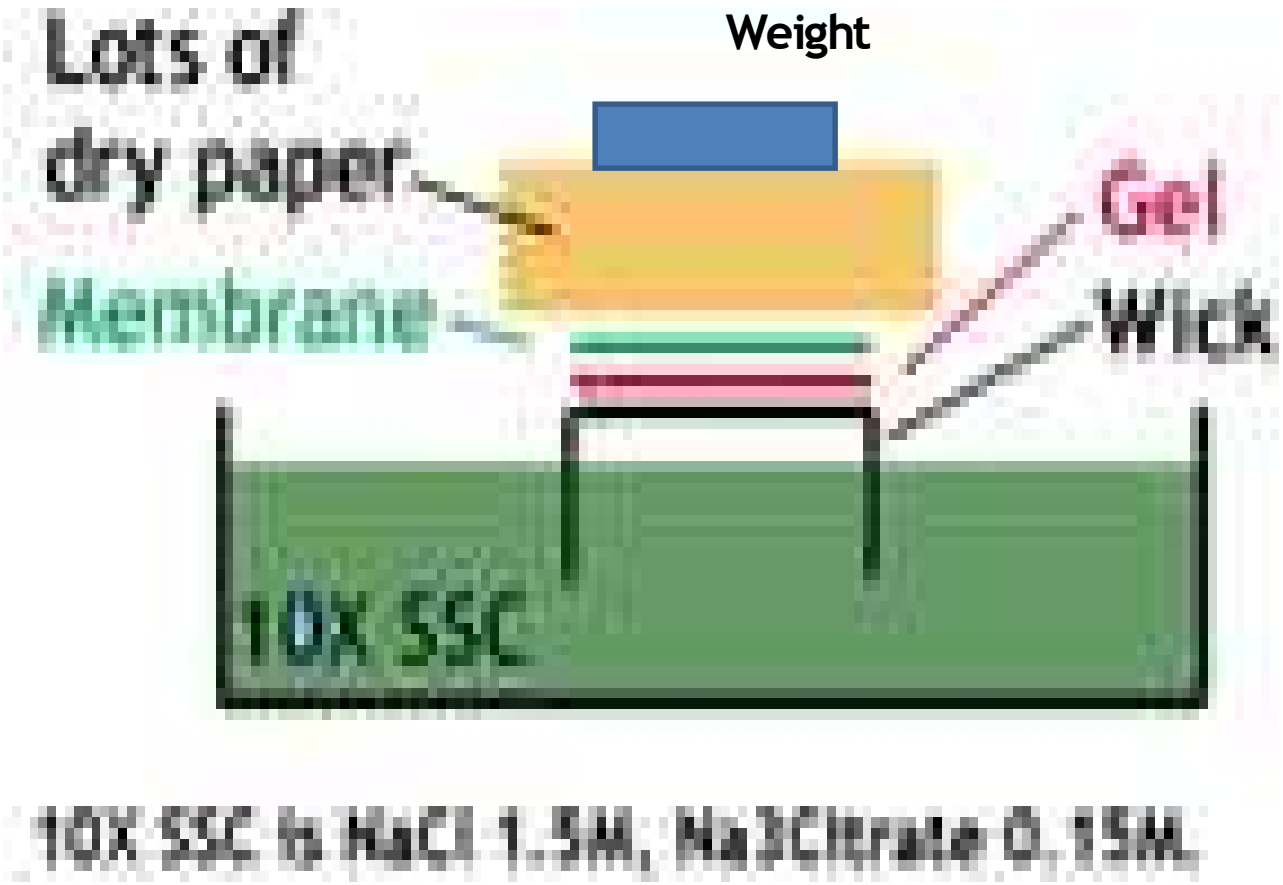
Sir Edwin Mellor Southern ,Fellow Royal Society and Trinity won Albert Lasker Award (2005) in Clinical Medical Research for the invention of the Southern Blot in 1975 when he was working as Professor of Biochemistry at the University of Edinburgh ,which is now a common molecular biology procedure to identify DNA sequence

Other blotting methods that employ similar principles but using RNAor Protein are named as Northern Blot and Western Blot in reference to original Southern Blot

In Northern hybridization (Blot) RNAs are transferred from gel to DBM (Diazobenzyl oxy methyl) paper or Nylon membrane and are fixed by baking. Denaturation step is not needed and the probe used for hybridization is single stranded DNA Since the base-pairing is in a sense the reverse of a *"southern*" experiment, this technique is referred to as a "*northern* blot".

The Western blot (Protein immunoblot) is widely used analytical technique to detect specific proteins in the tissue homogenate or extract. Proteins after extraction are separated by gel electrophoresis based on length of polypeptide. Proteins are transferred to a membrane e.g. Nitrocellulose or Poly vinyl di fluoride (PVDF) and are probed by using specific antibodies specific to target protein or antigen. The technique is also called immunoblotting and is to identify

BLOTTING ARRANEMENT



TRANSFER OF FRAGMENTS FROM GEL TOMEMBRANE

^{32}P ATP is used to label the probe radioactively. After hybridization, excess or unhybrized probe is washed from the membrane in several changes of buffer The wash can be with low or high stringency which can remove hybridization solution and also unused probes

The result is that only fully hybridized labeled probe molecules, with complementary sequence to the region of interest, remain bound.

A pre-hybrdization step is required before hybridization to block non-specific sites, to prevent single-stranded probe binding just anywhere on the membrane.

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