VRIJE UNIVERSITEIT BRUSSELS
INTERUNIVERSITY PROGRAMME MOLECULAR BIOLOGY

GENERAL PRACTICAL COURSE

SOUTHERN BLOTTING TECHNIQUE

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SOUTHERN BLOT

I. INTRODUCTION

II. PRINCIPLE

III. PROTOCOL

IV. APPLICATIONS

I. INTRODUCTION

- A technique which allows the detection of a specific DNA sequence (gene or other) in a large, complex sample of DNA (e.g. cellular DNA).

- Named after its inventor and developer, the British biologist Edwin M. Southern in 1975.

II. PRINCIPLE

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.

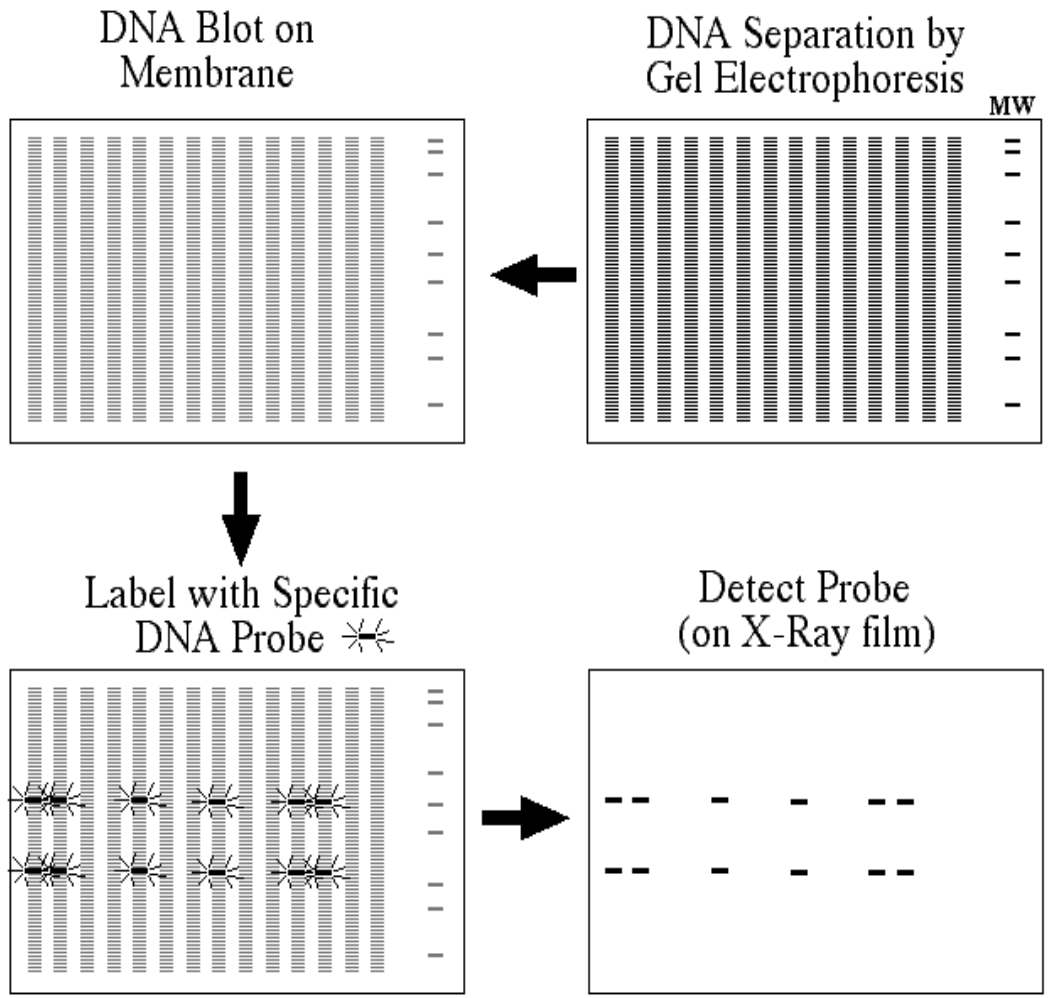
III. PROTOCOL

Step 1
DNA separation

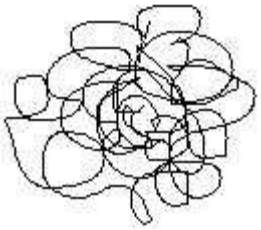
Step 2
Blot on membrane

Step 3
Label with specific DNA probe

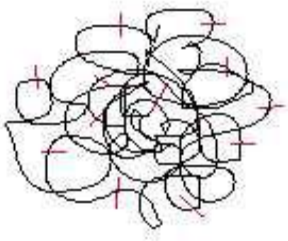
Step 4
Detect probe



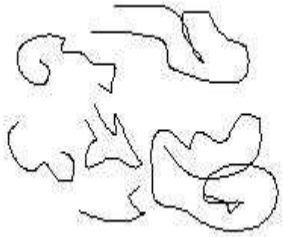
Step 1: DNA separation



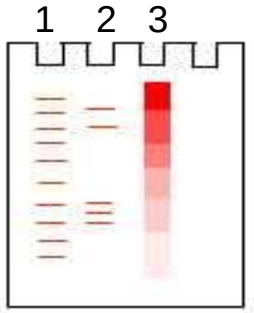
DNA is extracted from the cells and purified



DNA is restricted with enzymes



A large piece of DNA is chopped into smaller pieces using RE



Loading and running DNA agarose gel

Lane 1 – DNA marker
Lane 2 - Restricted DNA
Lane 3 - Unrestricted (whole) DNA

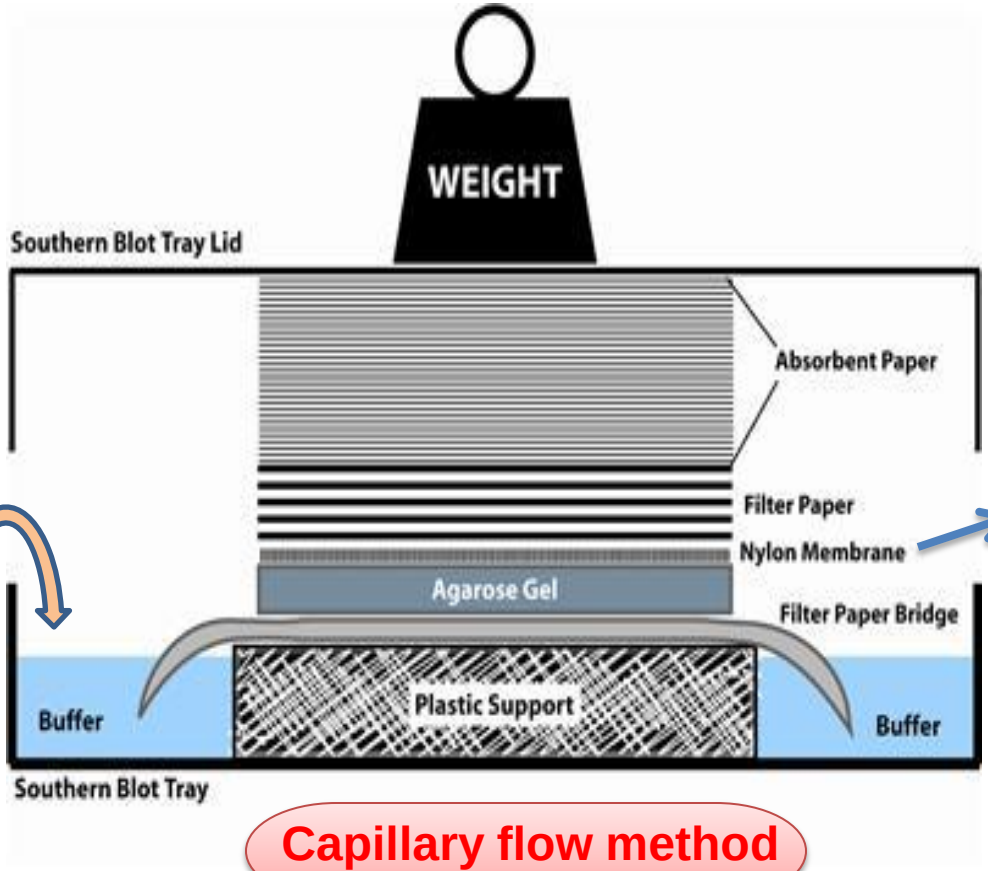
Step 2: Blot on membrane

Denature double-stranded DNA into single strands by incubation with NaOH

Transfer DNA to membrane

20X SSC buffer

12-16 hours

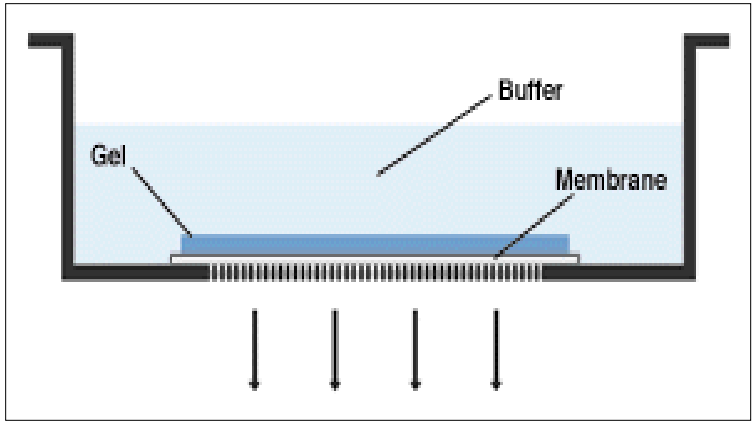


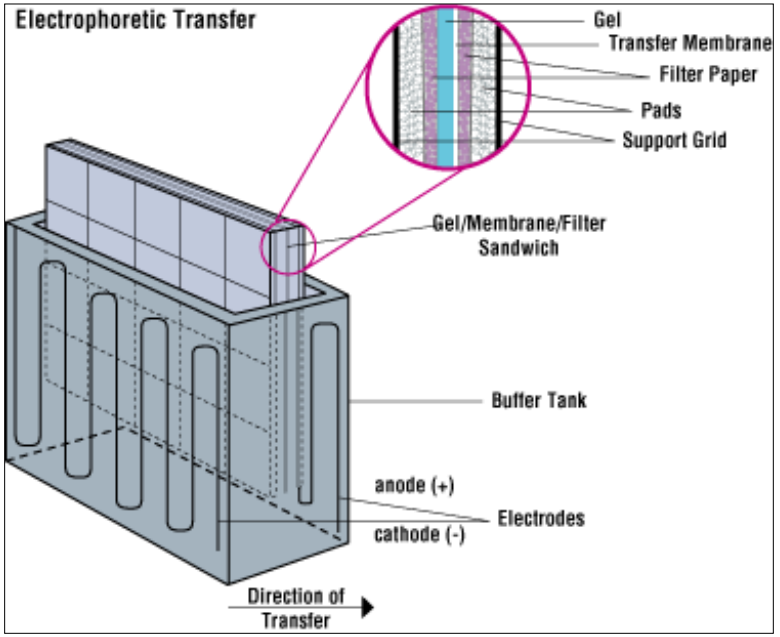
Nylon or Nitrocellulose

Capillary flow method

Step 2: Blot on membrane (cont)

Two other methods for transferring DNA to a membrane





Vacuum blotting

- Quite fast (1-3 hours)
- Gives excellent recoveries

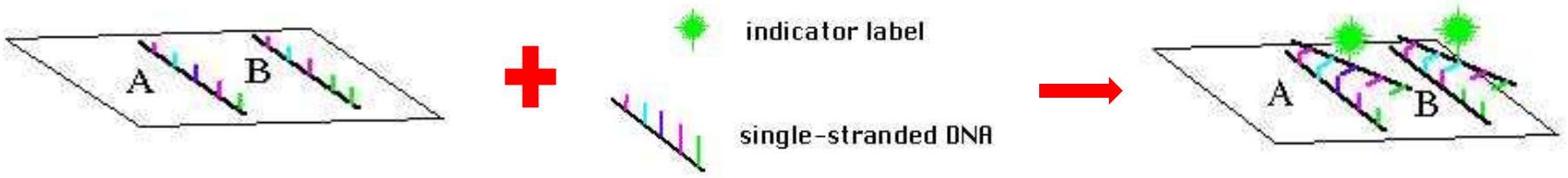
Electroblotting

- More popular for polyacrylamide gels

Step 3: Label with specific DNA probe

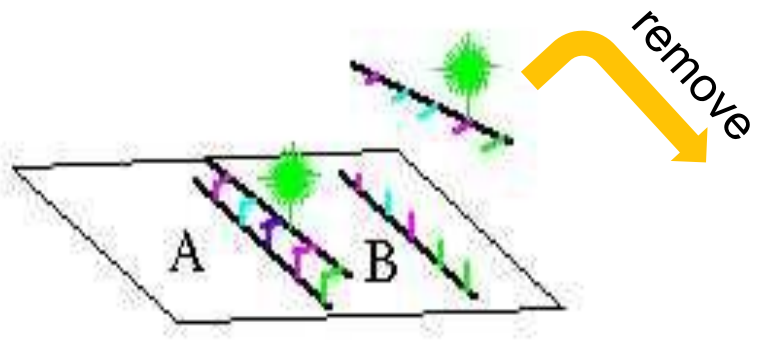
Attach the transferred DNA to the membrane by high temperature or UV

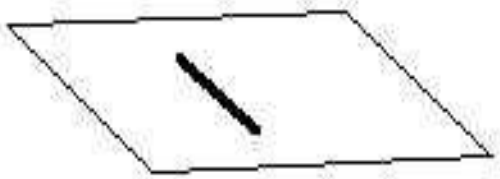
Incubate the blot with specific probe



* A probe is a small (25-2000 bp) length of DNA or RNA
  - Complementary to the sequence (gene) of interest
  - Labeled by incorporating radioactivity or tagging with a fluorescent or chromogenic dye

Step 4: Detect probe





Unbound probe is washed away from the membrane
(in 2X SSC buffer + 0.1% SDS)

Detect the pattern of hybridization

By autoradiography on X-ray film (in case of radioactive or fluorescent probe)

By development of color on the membrane (in case of chromogenic probe)

Example

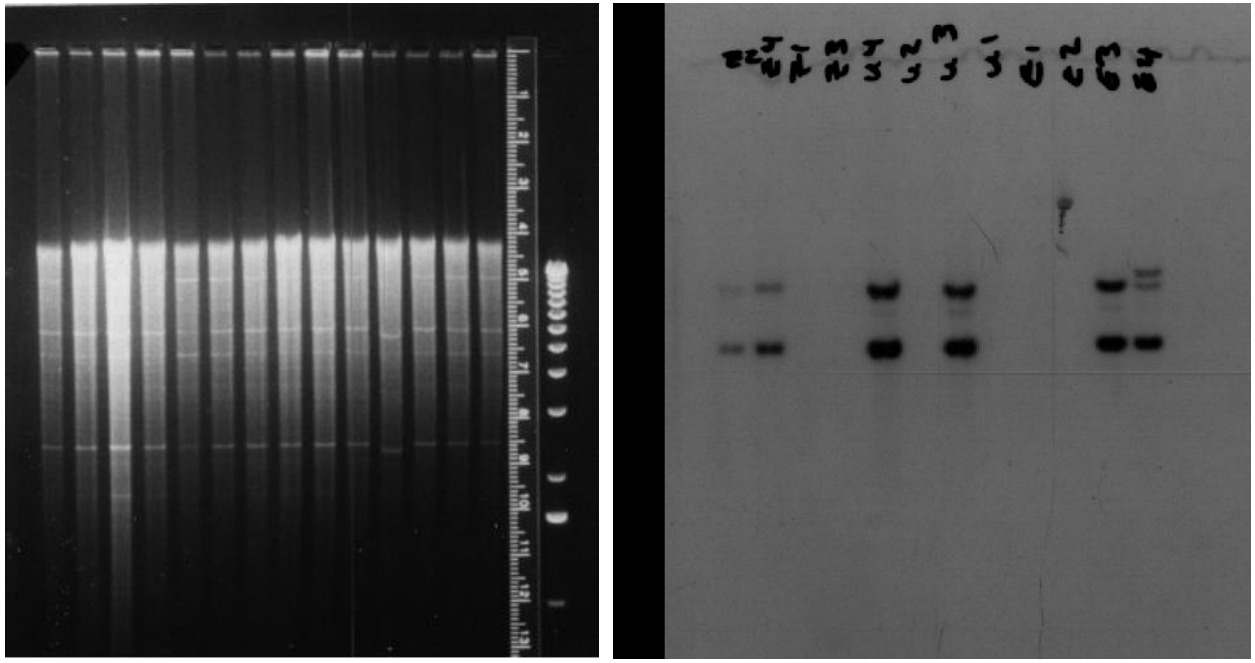
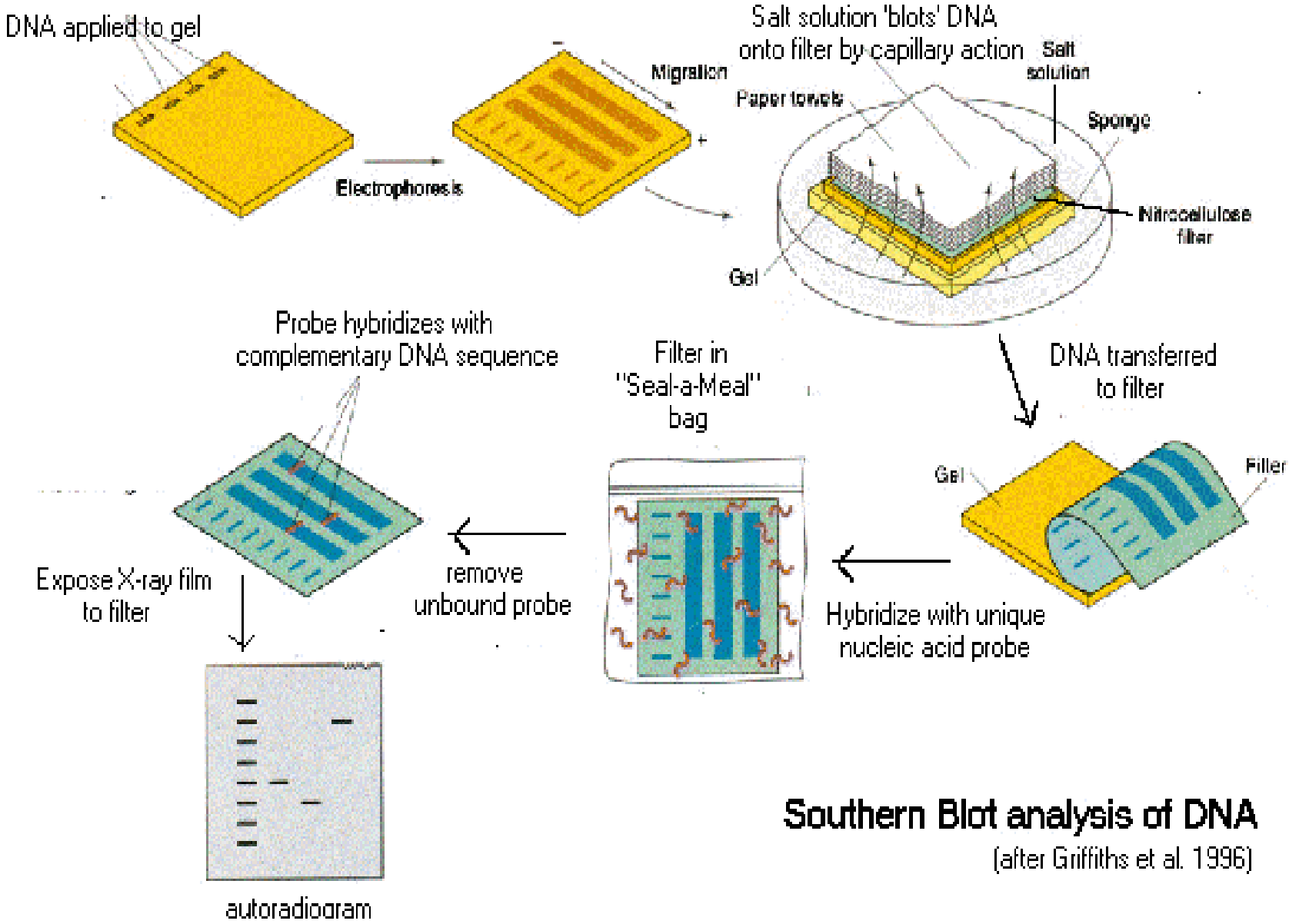


Figure. The figure on the left shows a photograph of a 0.7% agarose gel that has 14 different samples loaded on it. Each sample of DNA has been digested with the same restriction enzyme (EcoRI). The DNA was transferred to nitrocellulose and then probed with a radioactive fragment of DNA that was derived from the transformed gene. The figure on the right is a copy of the X-ray film and reveals which strains contain the target DNA and which ones do not.

SUMMARY OF SOUTHERN BLOT PROTOCOL



IV. APPLICATION

SB is used in gene discovery and mapping, evolution and development studies, diagnostics and forensics:

- Determine the molecular weight of a restriction fragment and to measure relative amounts in different samples
- Detect the presence of a particular bit of DNA in a sample
- Analyze the genetic patterns which appear in a person's DNA
- Analyze restriction digestion fragmentation of DNA or a biological sample
- In regards to genetically modified organisms
- Detect RFLP and VNTRs

References

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- http://www.bio.davidson.edu/courses/genomics/.../Southernblot.html
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THANK YOU FOR ATTENTION !