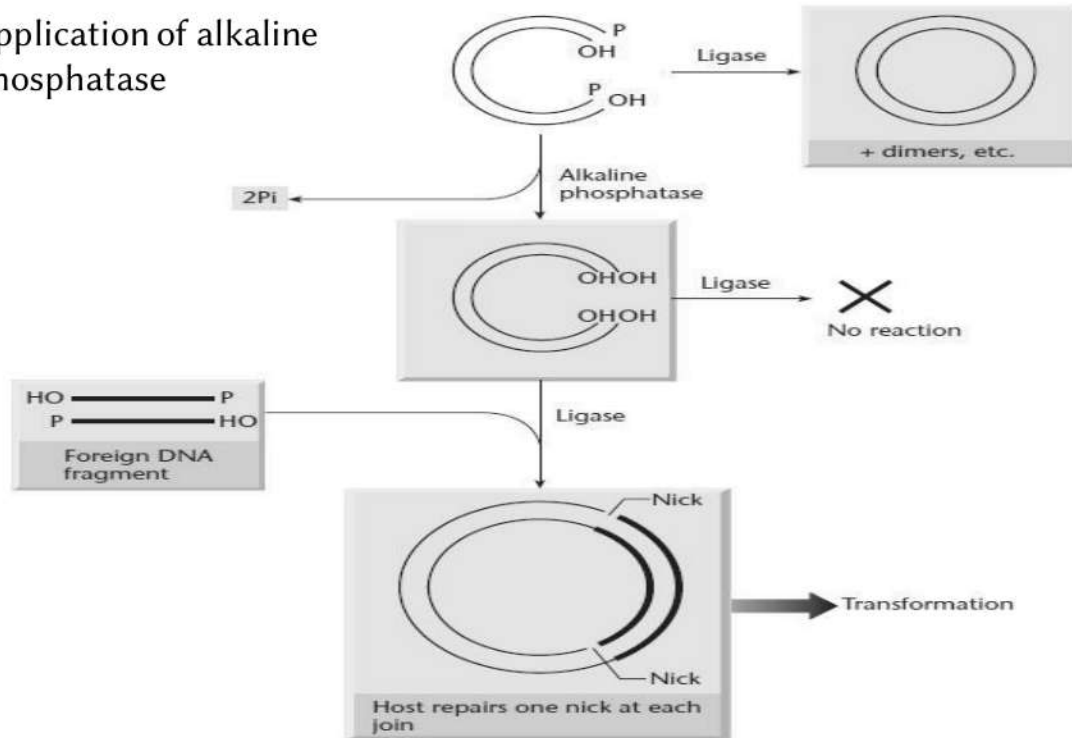


Application of alkaline phosphatase



1/8

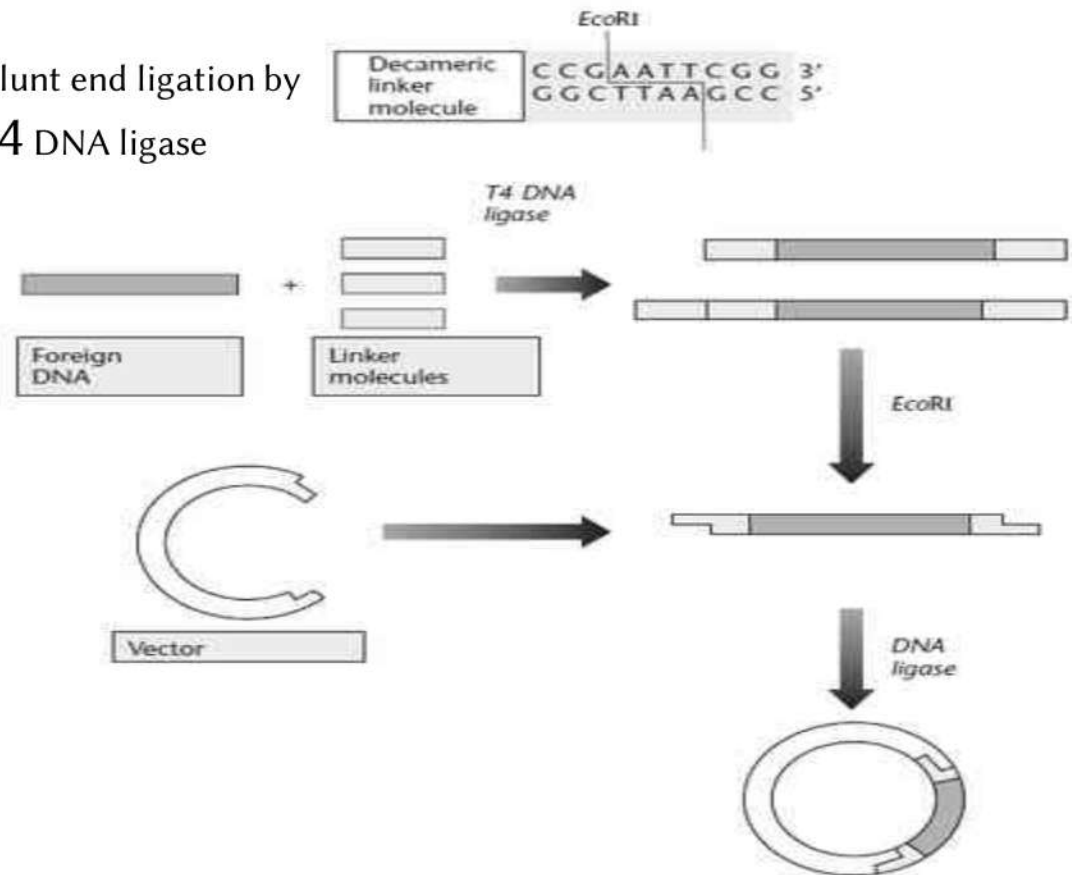
Joining DNA molecules

Lesson No.44

Blunt end ligation via linker molecules

- Joining DNA fragments with cohesive ends by DNA ligase is a relatively efficient process
- T4 DNA ligase has been used to joint blunt-ended DNA molecules

Blunt end ligation by
T4 DNA ligase



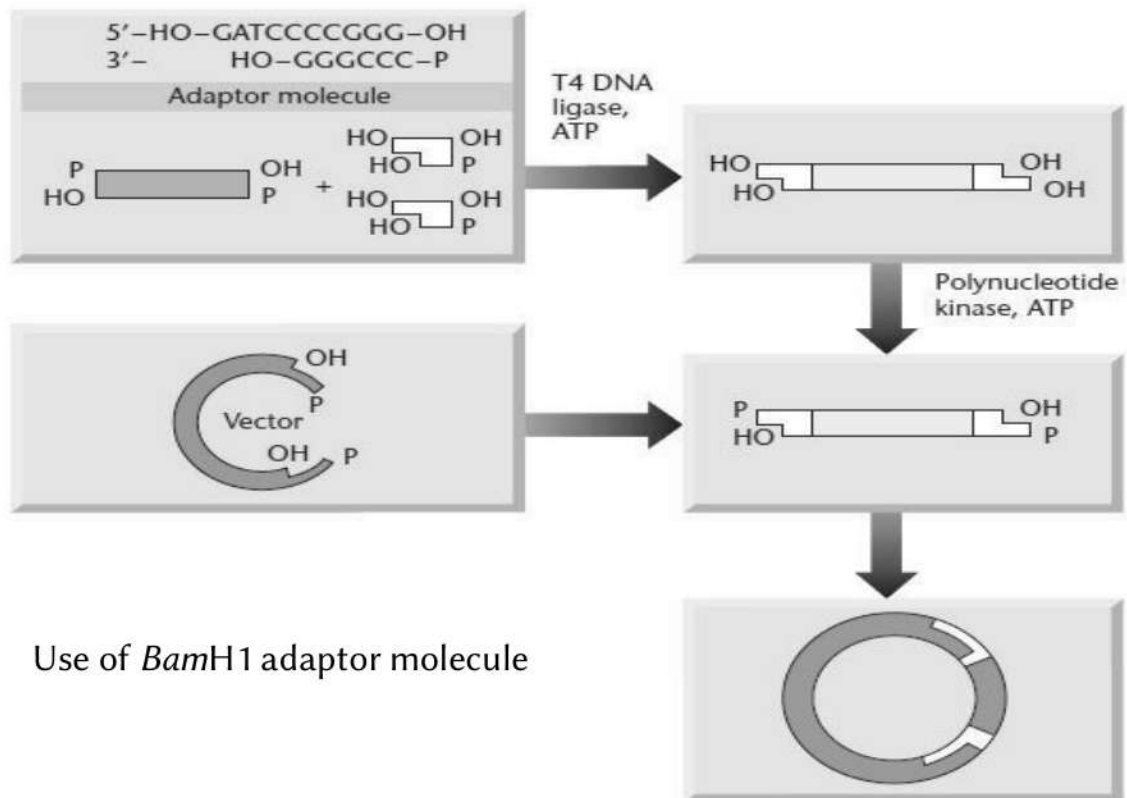
1/8

Joining DNA molecules

Lesson No.45

Adaptors

- Chemically synthesized adaptor molecules which have a preformed cohesive end can be used to insert blunt ended foreign DNA into vector



1/8

Joining DNA molecules

Adaptors

- The main difference between linker and adaptor is that former having blunt ends while later have one cohesive end

Essentials of Genetics

Lesson No.46

SOUTHERN BLOTTING

Southern Blotting

SOUTHERN BLOTTING

- The Southern blot is used to detect the presence of a particular piece of DNA in a sample by a molecular probe.

Southern Blotting

SOUTHERN BLOTTING

- Southern Blotting is named after its inventor, the British Biologist Edwin Southern (1975).

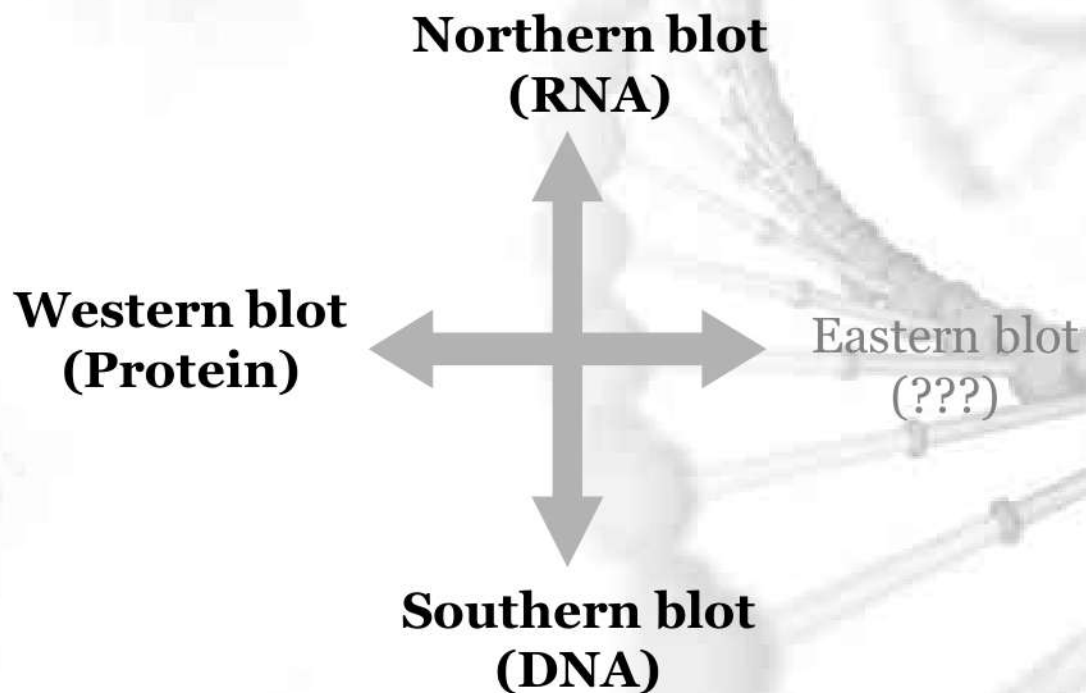
Southern Blotting

OTHER BLOTTING METHODS

- Other blotting methods with similar principles, but using protein or RNA, have been named in reference to Edwin Southern's name.

Southern Blotting

BACKGROUND



Southern Blotting

SOUTHERN BLOTTING

- Identify DNA sequence (gene) of interest.
- Identified DNA may be a small piece of DNA or a mutation.

Essentials of Genetics

Lesson No.47

SOUTHERN BLOTTING PROCEDURE

Southern Blotting - Procedure

PROCEDURE

- DNA is extracted from cells, leukocytes.
- DNA is cleaved into many fragments by restriction enzyme (e.g, BamH1, EcoR1 etc)
- The resulting fragments are separated on the basis of size by electrophoresis.
- The DNA fragments are denatured and transferred to nitrocellulose membrane for analysis.

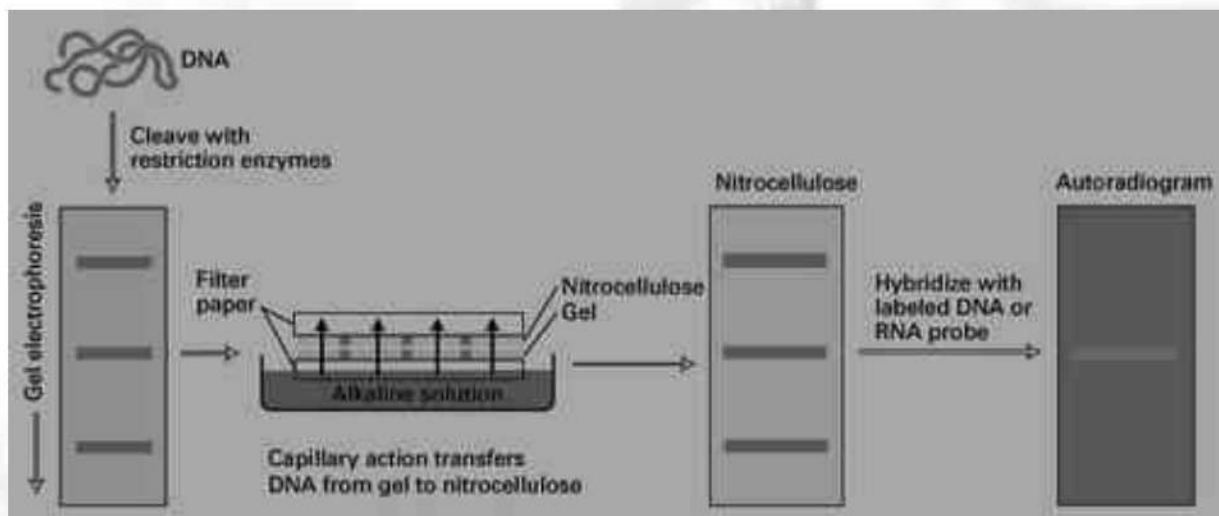
Southern Blotting - Procedure

PROCEDURE

- The labeled probe is added to the blocked membrane in buffer and incubated for several hours to allow the probe molecules to find their targets.
- Blot is incubated with wash buffers containing NaCl and detergent to wash away excess probe.
- Radioactive probes enable autoradiographic detection.

Southern Blotting - Procedure

PROCEDURE



Southern Blotting - Procedure

CONCLUSION

- Southern blot is used to detect the presence of a particular piece of DNA in a sample by a molecular probe.

Essentials of Genetics

Lesson No.48

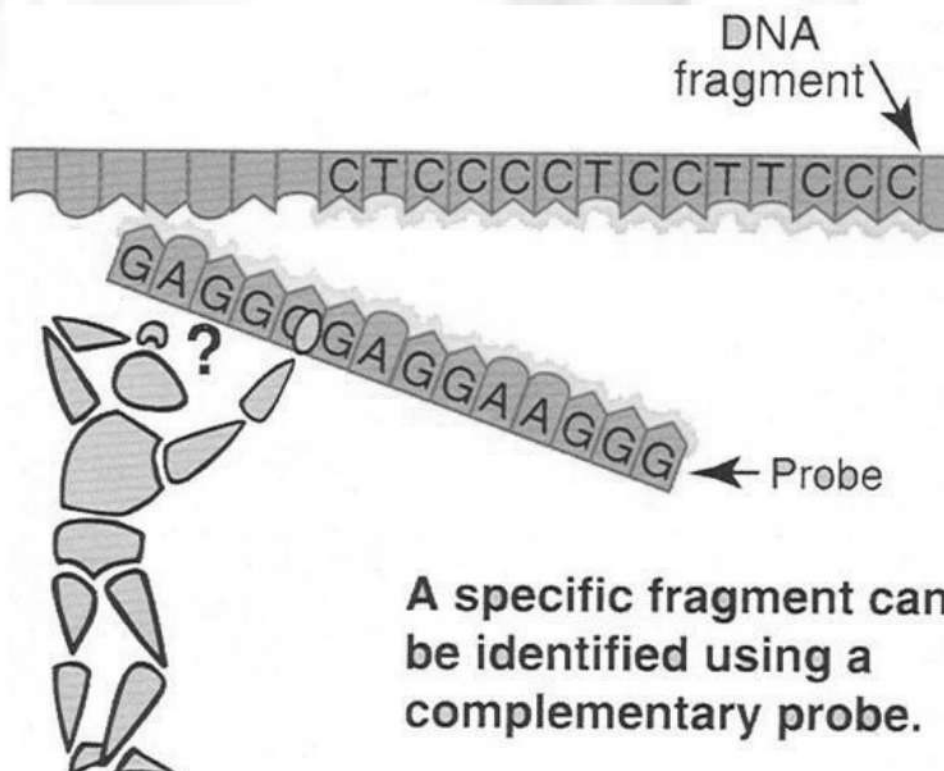
SOUTHERN BLOTTING PROBES

Southern Blotting - Probes

PROBES

- Labeled material to detect a target.
- For DNA: 20-30 nucleotides, complementary to a region in the gene or DNA.

Southern Blotting - Probes



Southern Blotting - Probes

RADIOACTIVE PROBE - P32

- Sensitive
- Relatively cheap
- Hazardous
- Radioactive waste disposal regulations should be followed

Southern Blotting - Probes

NON-RADIOACTIVE PROBE - BIOTIN

- Sensitive
- Relatively expensive

Southern Blotting - Probes

HYBRIDIZATION OF PROBES

- The binding between single stranded labeled probe to a complementary nucleotide sequence on the target DNA.

Southern Blotting - Probes

PROBES

- Labeled material to detect complementary region in the gene or DNA.

Essentials of Genetics

Lesson No.49

TRANSFER METHODS IN SOUTHERN BLOTTING

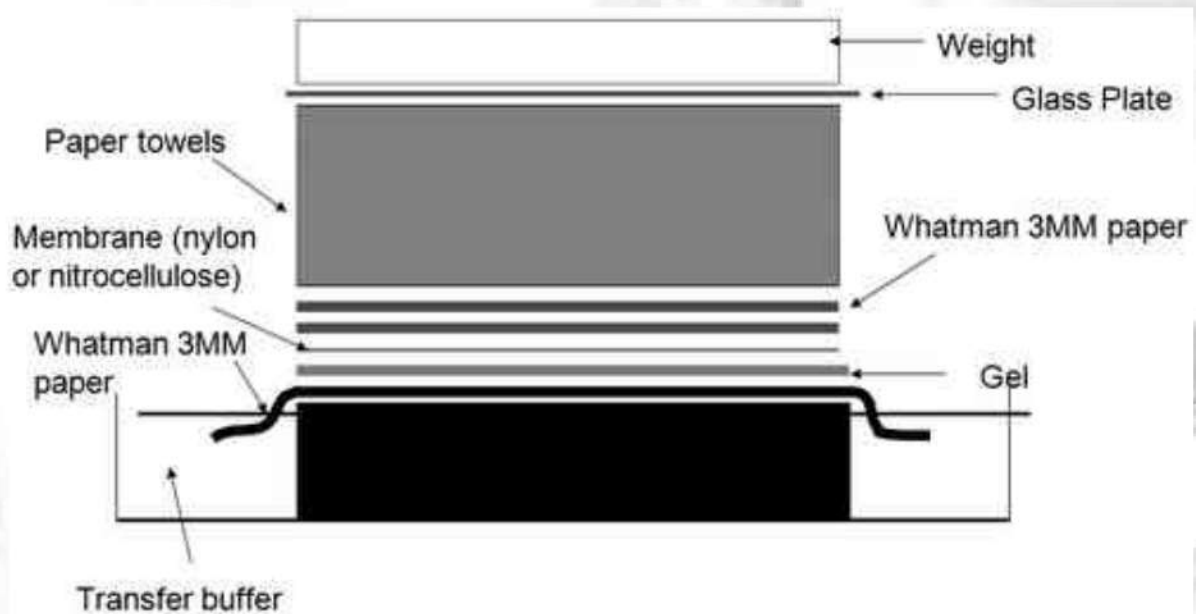
Transfer Methods - Southern

TRANSFER METHODS OF DNA TO MEMBRANE

- Upward capillary transfer
- Downward capillary transfer
- Simultaneous transfer to two membranes
- Electrophoretic transfer
- Vacuum transfer

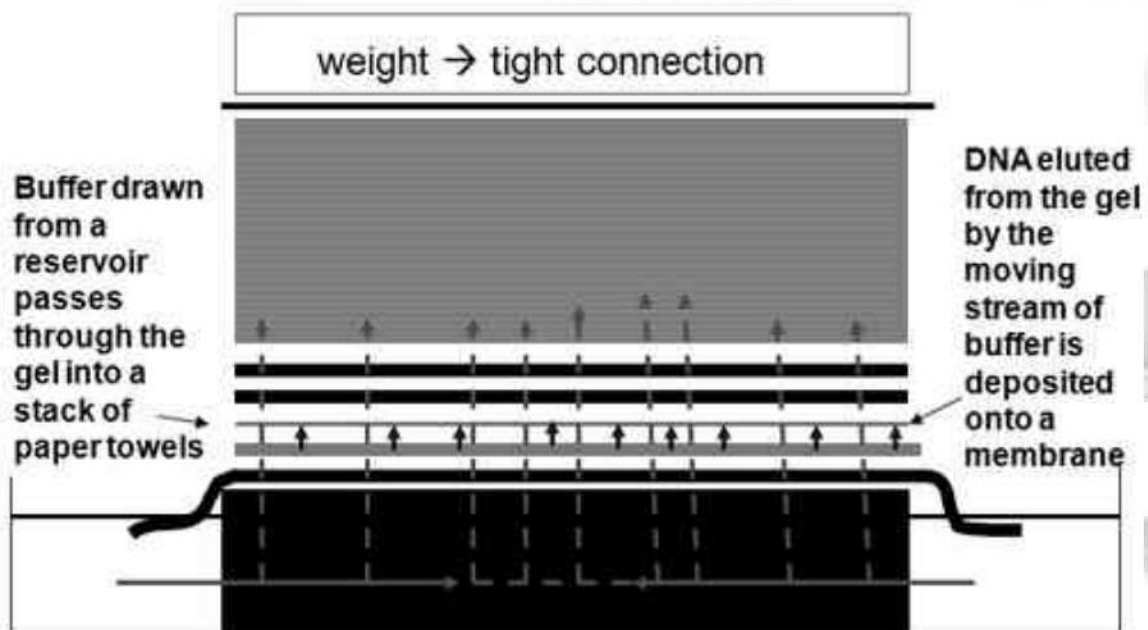
Transfer Methods - Southern

UPWARD TRANSFER



Transfer Methods - Southern

UPWARD TRANSFER OF DNA TO MEMBRANE



Transfer Methods - Southern

TRANSFER METHODS

- Upward capillary transfer
- Downward capillary transfer
- Simultaneous transfer to two membranes
- Electrophoretic transfer
- Vacuum transfer

Essentials of Genetics

Lesson No.50

MUTATIONS IDENTIFICATION BY SOUTHERN BLOTTING

Mutations Identification

MUTATION IDENTIFICATION

- The presence of a mutation affecting a restriction site causes the pattern of bands to differ from those seen in a normal gene.
- A change in one nucleotide may alter the nucleotide sequence so that the restriction endonuclease fails to recognize and cleave at that site.

Mutations Identification

MUTATION IDENTIFICATION

- Digestion of genomic DNA to DNA fragments.
- Size-separation of the fragments.
- In situ denaturation of the DNA fragments.
- Transfer of denatured DNA fragments into a solid support (nylon or nitrocellulose).
- Hybridization of the immobilized DNA to a labeled probe (DNA, RNA).
- Detection of the bands complementary to the probe (e.g. by autoradiography).

Mutations Identification

MUTATION IDENTIFICATION

- Estimation of the size & number of the bands generated after digestion of the genomic DNA will be different.

Mutations Identification

MUTATION IDENTIFICATION

- Mutations can be identified by Southern blotting.

Essentials of Genetics

Lesson No.51

NORTHERN BLOTTING

Northern Blotting

NORTHERN BLOTTING

- Northern blot is a method used for detection of a specific RNA sequence in RNA samples.

Northern Blotting

PROCEDURE

- Isolation of intact mRNA.
- Separation of RNA according to size (through a denaturing agarose gel).
- Transfer of the RNA to a solid support.
- Fixation of the RNA.
- Hybridization of the immobilized RNA to probes complementary to the sequences of interest.

Northern Blotting

PROCEDURE

- Removal of probe molecules that are nonspecifically bound to the solid matrix.
- Detection, capture and analysis of an image of the specifically bound probe molecules.

Northern Blotting

NORTHERN BLOTTING

- Northern blot is a method used for detection of a specific RNA sequence in RNA samples.

Essentials of Genetics

Lesson No.52

NORTHERN BLOT APPLICATIONS

Northern Blot - Applications

APPLICATIONS

- Study of gene expression in eukaryotic cells.
- To measure the amount & size of RNAs transcribed from eukaryotic genes.
- To estimate the abundance of RNAs.

Northern Blot - Applications

APPLICATIONS

- To equalize the amounts of RNA loaded into lanes of gels.
- Use of housekeeping gene (endogenous constitutively-expressed gene).
- Normalizing samples according to their content of mRNAs of the housekeeping gene.

Northern Blot - Applications

APPLICATIONS

- Northern blot is used in many ways while studying RNAs.

Essentials of Genetics

Lesson No.53

WESTERN BLOTTING

Western Blotting

WESTERN BLOTTING

- A technique used to detect the presence of a specific protein in a complex protein mixture.

Western Blotting

WESTERN BLOTTING

- To determine the molecular weight of a protein.
- To measure relative amounts (quantitation) of the protein present in complex mixtures of proteins that are not radiolabeled.

Western Blotting

WESTERN BLOTTING

- Western blots have become one of the most common analytical tools for the detection of viral proteins.
- Characterization of monoclonal and polyclonal antibody preparations and in determining the specificity of the immune response to viral antigens.

Western Blotting

WESTERN BLOTTING

- A technique used to detect the presence of a specific protein in a complex protein mixture.

Essentials of Genetics

Lesson No.54

WESTERN BLOTTING PROCEDURE

Western Blotting - Procedure

PROCEDURE

- 1- Sample preparation
- 2- Gel Electrophoresis
- 3- Blotting (or transfer)
- 4- Blocking
- 5- Antibody probing
- 6- Detection

Western Blotting - Procedure

PROCEDURE

1- The choice of extraction method depends primarily on the sample and whether the analysis is targeting all the proteins in a cell or only a component from a particular subcellular fraction.

2- Samples are loaded into separate wells. A protein marker is also loaded. The separated protein mixtures are transferred to a solid support for further analysis.

Western Blotting - Procedure

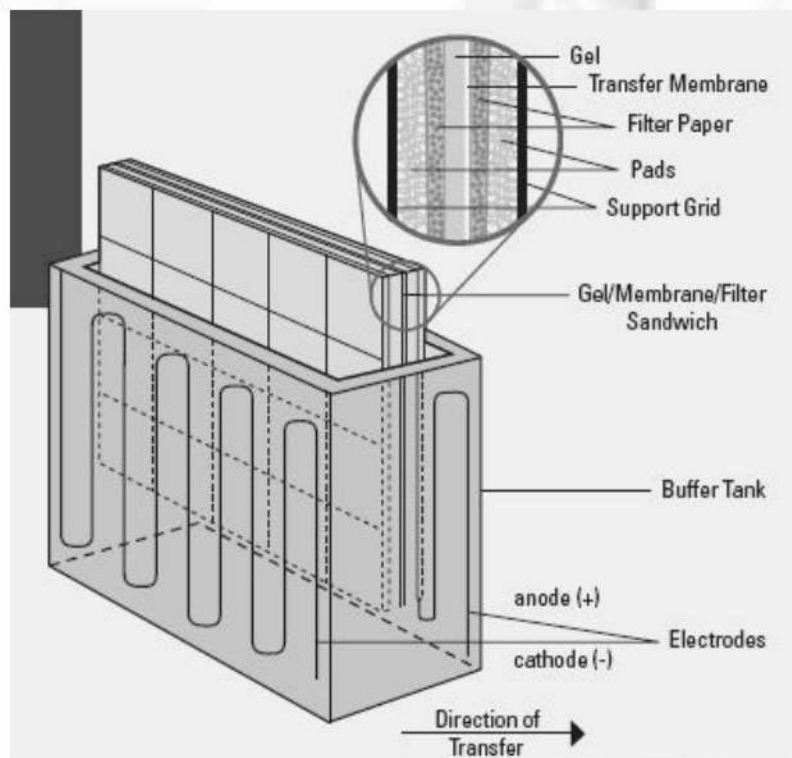
PROCEDURE

3- Transfer can be done in wet or semi-dry conditions. Semi-dry transfer is generally faster. Wet transfer is recommended for large proteins, >100 kD.

4- Blocking is a very important step in the immunodetection phase of Western blotting because it prevents non-specific binding of antibody to the blotting membrane.

Western Blotting - Procedure

ELECTROPHORETIC TRANSFER



Western Blotting - Procedure

PROCEDURE

Protein of interest is detected and localized using a specific antibody. Western blotting protocols utilize a non-labeled primary antibody directed against the target protein.

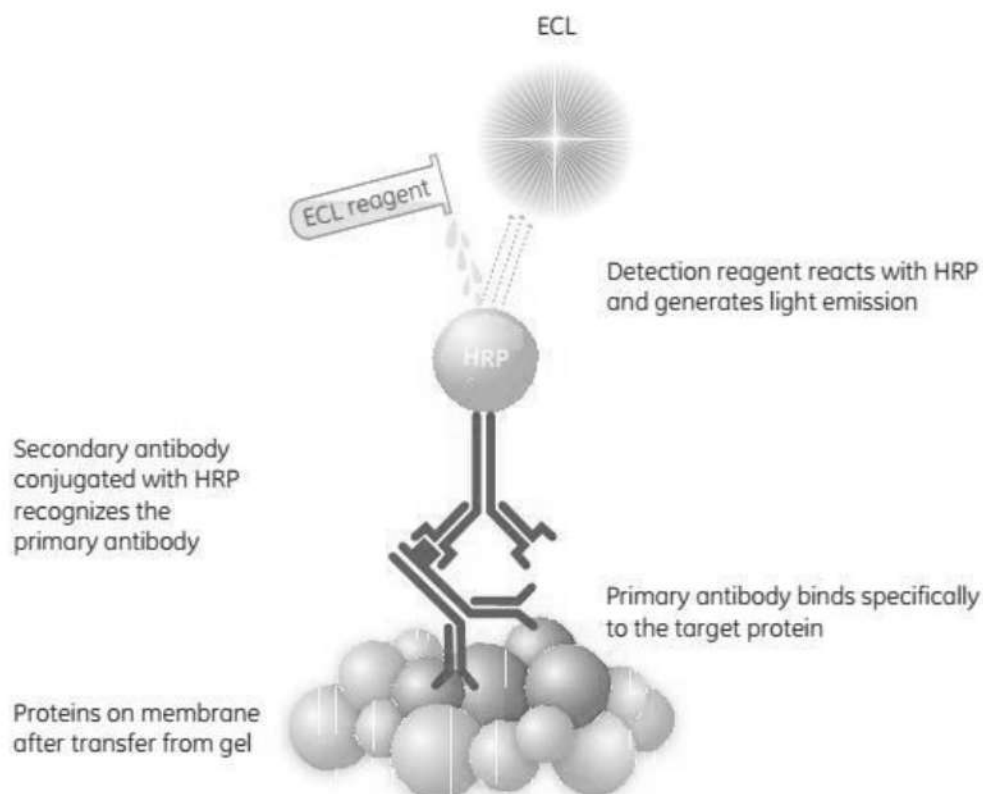
A species-specific, labeled secondary antibody directed against the constant region of the primary antibody is then used. The most common antibody label used in Western blots is HRP.

Western Blotting - Procedure

PROCEDURE

The signal is detected when HRP is exposed to a substrate solution in the final step of the immunodetection procedure

Western Blotting - Procedure



Western Blotting - Procedure

WESTERN BLOTTING

A molecular technique
to study proteins.

Lesson No.55

**Applications of
western blotting**

- Analysis Of IgG Fractions purified from human plasma.
- Diagnosis of HIV by ELISA, involves the western blotting technique.
- Western blotting technique is also used to Detect Some Forms Of Lyme Disease.
- Western blotting technique is used in Definitive Test For BSE, which is commonly know as Mad cow disease.
- Confirmatory Test For Hepatitis-B involves western blotting technique.
- Western blotting test is used in the Analysis Of Biomarkers such as hormones, growth factors & cytokines.
- This technique is also employed in The Gene Expression Studies

Limitations in western blotting

- very delicate and time consuming process. A minute imbalance at any level of the procedure can skew the results of the entire process.
- Incorrect labeling of the protein can happen due to the reaction of secondary antibody.
- Cause erroneous in bands or no bands due to insufficient transfer.
- Well trained technicians are required for this technique.
- Primary antibody availability is crucial.
- It is just a semi-quantitative at best. Only an approx. estimation & not a precise measurement of molecular weight of the protein is possible

Conclusion

- Western blotting technique is simply a way to identify unknown proteins on a polyacrylamide gel.
- It is sometimes called as protein blotting or immunoblotting.
- It is a widely used analytical technique in the fields of molecular biology, immunogenetics, and other biochemistry disciplines.
- Western blotting technique is also used in the field of medical diagnostics. i.e., in the analysis of various kinds of diseases.
- Apart from the limitations of western blotting, it is more helpful now a days. Hence, The discovery of Western blotting technique has become boon in the field of science & technology

Lesson No.56

**Difference
between different
blotting technique**

Northern vs Southern vs Western Blotting

Type of Molecule Detected	
Northern Blotting	Northern blotting detects a specific RNA sequence from an RNA sample.
Southern Blotting	Southern blotting detects a specific DNA sequence from a DNA sample.
Western Blotting	Western blotting detects a specific protein from a protein sample.
Type of Gel	
Northern Blotting	This uses Agarose/formaldehyde gel.
Southern Blotting	This uses an Agarose gel.
Western Blotting	This uses Polyacrylamide gel.
Blotting Method	
Northern Blotting	This is a capillary transfer.
Southern Blotting	This is a capillary transfer.
Western Blotting	This is an electric transfer.

Northern vs Southern vs Western Blotting

Probes Used	
Northern Blotting	cDNA or RNA probes labeled radioactively or nonradioactively.
Southern Blotting	DNA probes are labeled radioactively or non-radioactively.
Western Blotting	Primary antibodies are used as probes.
Detection System	
Northern Blotting	This is done using an autoradiograph, or detection of light or color change.
Southern Blotting	This is done using an autoradiograph, detection of light or color change.
Western Blotting	This is done using the detection of light or color change.

Lesson No.57

Single nucleotide polymorphism

What is SNP ?

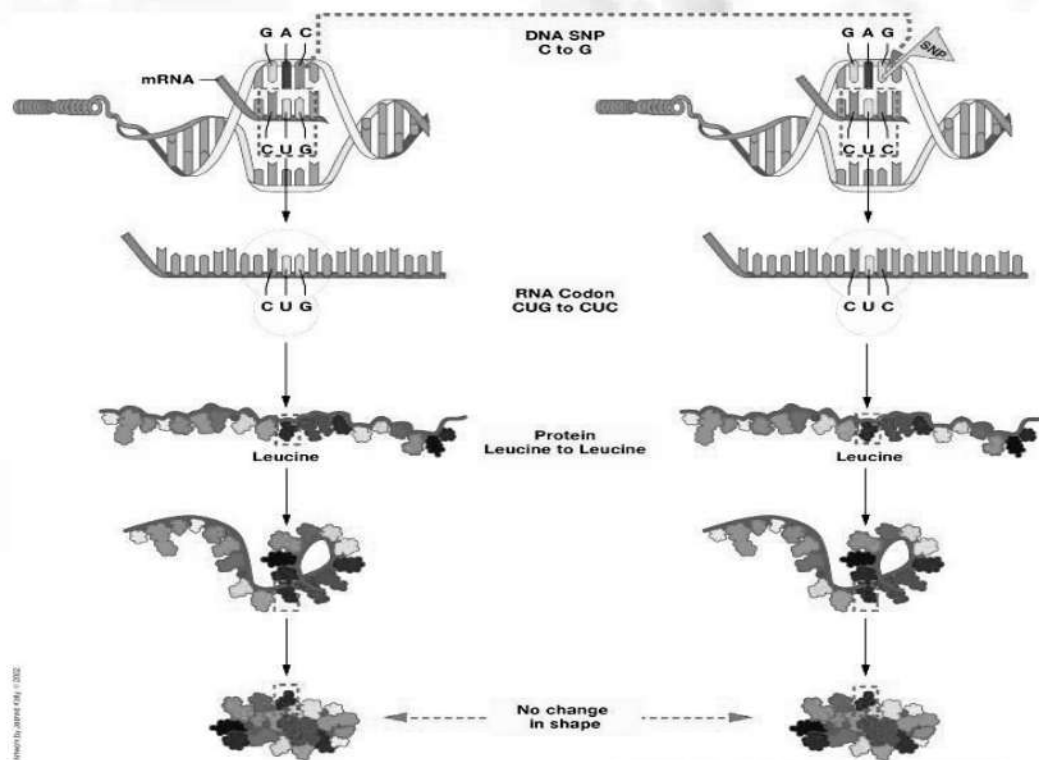
- A **SNP** is defined as a single base change in a DNA sequence that occurs in a significant proportion (more than 1 percent) of a large population.

Some Facts

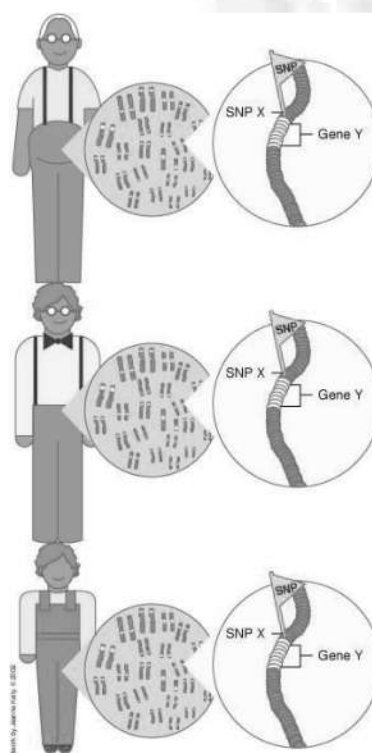
- In human beings, 99.9 percent bases are same.
- Remaining 0.1 percent makes a person unique.
 - Different attributes / characteristics / traits
 - ✦ how a person looks,
 - ✦ diseases he or she develops.
- These variations can be:
 - Harmless (change in phenotype)
 - Harmful (diabetes, cancer, heart disease, Huntington's disease, and hemophilia)
 - Latent (variations found in coding and regulatory regions, are not harmful on their own, and the change in each gene only becomes apparent under certain conditions e.g. susceptibility to lung cancer)

- ▶ SNPs are found in
 - coding and (mostly) noncoding regions.
- ▶ Occur with a very high frequency
 - about 1 in 1000 bases to 1 in 100 to 300 bases.
- ▶ The abundance of SNPs and the ease with which they can be measured make these genetic variations significant.
- ▶ SNPs close to particular gene acts as a marker for that gene.
- ▶ SNPs in coding regions may alter the protein structure made by that coding region.

SNPs may / may not alter protein structure



SNPs act as gene markers



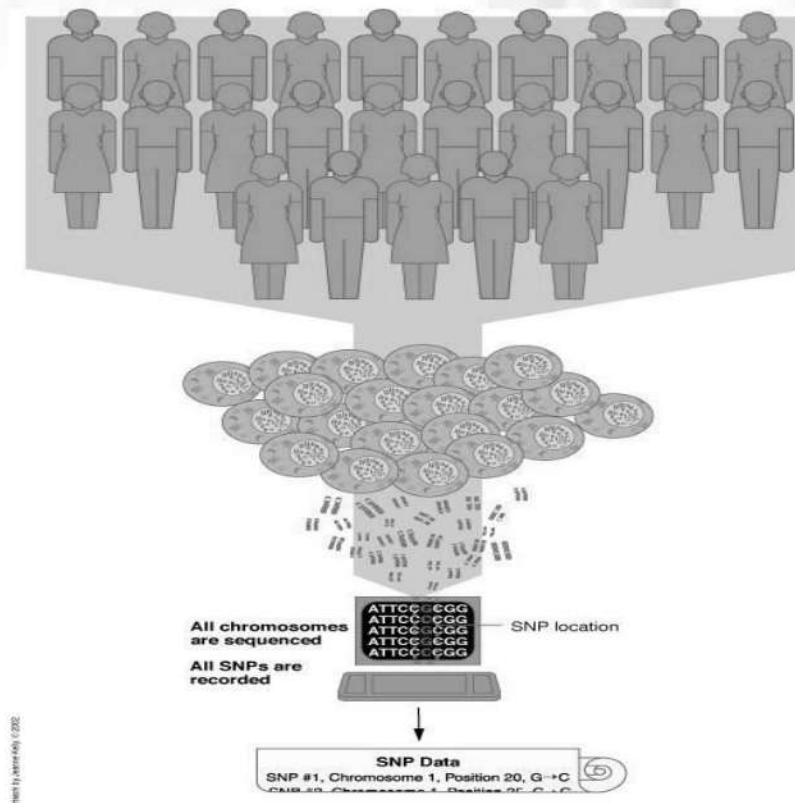
Lesson No.58

Single nucleotide polymorphism-2

SNP maps

- ▶ Sequence genomes of a large number of people
- ▶ Compare the base sequences to discover SNPs.
- ▶ Generate a single map of the human genome containing all possible SNPs => SNP maps

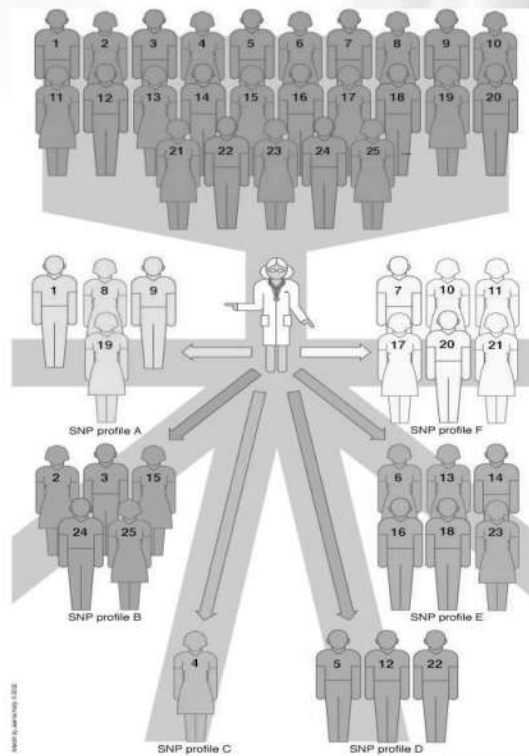
SNP Maps



SNP Profiles

- Genome of each individual contains distinct SNP pattern.
- People can be grouped based on the SNP profile.
- SNPs Profiles important for identifying response to Drug Therapy.
- Correlations might emerge between certain SNP profiles and specific responses to treatment.

SNP Profiles



Lesson No.59

**How to detect
SNPs**

Techniques to detect known Polymorphisms

- Hybridization Techniques
 - Micro arrays
 - Real time PCR
- Enzyme based Techniques
 - Nucleotide extension
 - Cleavage
 - Ligation
 - Reaction product detection and display

Techniques to detect unknown Polymorphisms

- Direct Sequencing
- Microarray
- Cleavage / Ligation
- Electrophoretic mobility assays

Direct Sequencing

- ▶ Sanger dideoxysequencing can detect any type of unknown polymorphism and its position, when the majority of DNA contains that polymorphism.
- ▶ Misses polymorphisms and mutations when the DNA is heterozygous
- ▶ limited utility for analysis of solid tumors or pooled samples of DNA due to low sensitivity
- ▶ Once a sample is known to contain a polymorphism in a specific region, direct sequencing is particularly useful for identifying a polymorphism and its specific position.
- ▶ Even if the identity of the polymorphism cannot be discerned in the first pass, multiple sequencing attempts have proven quite successful in elucidating sequence and position information.

Lesson No.60

SNP Screening

SNP Screening

- ❖ Two different screening strategies
 - Many SNPs in a few individuals
 - A few SNPs in many individuals
- ❖ Different strategies will require different tools
- ❖ Important in determining markers for complex genetic states

○ SNP genotyping methods for detecting genes contributing to susceptibility or resistance to multifactorial diseases, adverse drug reactions:

○ => case-control association analysis

- case GCCGTTGAC... .
- GCCATTGAC... .
- Control ...GCCATTGAC... .
- GCCATTGAC... .
-



SIGNIFICANCE OF SNPs

- ❖ IN DISEASE DIAGNOSIS
- ❖ IN FINDING PREDISPOSITION TO DISEASES
- ❖ IN DRUG DISCOVERY & DEVELOPMENT
- ❖ IN DRUG RESPONSES
- ❖ INVESTIGATION OF MIGRATION PATTERNS

ALL THESE ASPECT WILL HELP TO LOOK FOR MEDICATION
& DIAGNOSIS AT INDIVIDUAL LEVEL

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PPT Slides 1 TO 66**

Regards : Zarva Chaudhary

Merged File date 2021

Admin:

** Zarva Chaudhary **

Chandhary Moazzam

** Laiba Maki **

Lesson No.61

SNP-Haplotype

HAPLOTYPE

- A set of closely linked genetic markers present
- on one chromosome which tend to be inherited
 - together (not easily separable by recombination)