

IN thE Name OF ALLAH
who is most Mercifull and
Kind

Essentials of Genetics

BIO203

Merged PPTs for Final term

Lecture No. 27
MINI-PRIMER PCR

Mini Primer PCR uses a thermostable polymerase (S-Tbr) that can extend from short primers as short as 9 or 10 nucleotides. This method permits PCR targeting to smaller primer binding regions, and is used to amplify conserved DNA sequences, such as the 16S (or eukaryotic 18S) rRNA gene.PCR that can extend from short primers

Lecture No. 28
ADVANTAGES AND LIMITATIONS OF PCR

ADVANTAGES

Specific, Simple, rapid, relatively inexpensive, Amplifies from low quantities
Works on damaged DNA Sensitive Flexible, Contamination risk, Primer complexities, Primer-binding site complexities, Amplifies rare species, Detection methods

Lecture No. 29

APPLICATIONS OF PCR
APPLICATIONS

Detection of Infectious diseases: AIDS, TB, CMV, H1N1, etc Viral, Bacterial and fungal infections,

Diagnosis of latent viruses. Forensic applications: DNA finger printing
Detection of Mutations: Inherited disorders & carriers Track DNA abnormalities . Prenatal diagnosis of genetic disorders. Detection of pathogens Pre-natal diagnosis DNA fingerprinting Gene therapy Mutation screening. Drug discovery, Classification of organisms Genotyping, Molecular Archaeology , Molecular Epidemiology Molecular Ecology, Bioinformatics Genomic cloning . Site-directed mutagenesis Gene expression studies

Lecture No. 30
Polymerase chain reaction (PCR) PCR-Gen cloning and expression

PCR has been used in gene cloning and screening of genomic libraries

Applications of PCR

PCR has widespread applications in various fields of life sciences including genetic engineering, medical, forensic, agriculture, environment etc

Lecture No. 31

Polymerase chain reaction (PCR) PCR-Medicine

PCR has major impact on medicine especially in the field of clinical microbiology or diagnosis

Molecular tools have also allowed to perform prenatal genetic diagnosis\

Lecture No. 32
Polymerase chain reaction (PCR)

PCR-Forensic sciences:

Forensic science is the application of scientific procedures to solve criminal and legal matters. Molecular methods are used to established the filiatiions of a person or to obtain evidence from minimal samples of saliva, semen or other tissues

PCR-DNA profiling:

DNA profiling or DNA fingerprinting is a forensic technique used to identify individuals by characteristics of their DNA

Lecture No. 33

Polymerase chain reaction (PCR)-PCR-Agricultural sciences and environment

PCR has also facilitated research in detection of pathogens in plants, animals and environment

Lecture No. 34

Polymerase chain reaction (PCR PCR-Agricultural sciences and environment

PCR has also facilitated research in detection of pathogens in plants, animals and environment

Lecture No. 35

Cutting DNA molecules Types of restriction and modification (R-M) system

Type I, Type II, Type III, Type IIs

Type I: Type I systems were the first to be characterized from *E. coli* K12

The active enzyme consists of two restriction subunit, two modification subunit and one recognition subunit. Type I systems are of little value for gene manipulation

Type II: Most of the useful R-M system is Type II. Type II enzymes recognize a defined sequence and cut within it

Type III: Type III enzymes have symmetrical recognition sequences but otherwise resemble type I systems and are of little value

Type IIs: Type IIs systems have similar cofactors and structure to type II but restriction occurs at a distance from recognition site that limits their usefulness.

Lecture No. 36

Cutting DNA molecules--Nomenclature : A suitable system was proposed by Smith and Nathans (1973). The species name of host organisms is identified by the first letter of genus and first two letters of specific epithet
E. coli = Eco, *H. influenzae* = Hin

Strain identification is written as EcoK . In case, host strain has several restriction and modification systems, these are identified by roman numerals, for example, in case of *H. influenzae*, *HindI*, *HindII*, *HindIII* etc. All restriction enzymes have general name endonuclease R and modification-methylase M followed by the system name, for example, in case of *H. influenzae*. R. *HindIII* or M. *HindIII* Examples of restriction endonuclease nomenclature

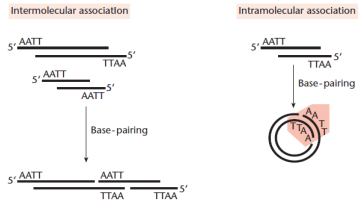
Enzyme	Enzyme source	Recognition sequence
<i>SmaI</i>	<i>Serratia marcescens</i> , 1st enzyme	CCCGGG
<i>HaeIII</i>	<i>Haemophilus aegyptius</i> , 3rd enzyme	GGCC
<i>HindII</i>	<i>H. influenzae</i> , strain d, 2nd enzyme	GTPyPuAC
<i>HindIII</i>	<i>H. influenzae</i> , strain d, 3rd enzyme	AAGCTT
<i>HamHI</i>	<i>Bacillus amyloliquefaciens</i> , strain H, 1st enzyme	GGATCC

Lecture No. 37
Cutting DNA molecules

Target sites

Type II endonucleases recognize and cleave DNA within particular sequences of four to eight nucleotides which have a twofold axis of rotational symmetry i.e. referred as *palindromes*

5'-GAATTC-3'
5'-CTTAAG-3'
5'-GAATTC-3'
3'-CTTAAG-5'
5'-G/AA*TT C-3'
3'-CTTA* A/ G-5'
5'-G 5'-AATTC-3'
3'-CTTAA-5' G-5'
Single stranded breaks by *EcoR1*



Lecture No. 38

Number and size of restriction fragments

The number and size of fragments generated by restriction enzyme depend on the frequency of occurrence of the target site in the DNA to be cut. Four base recognition site occurs every 4⁴ (256) bp
Six base recognition site occurs every 4⁶ (4096) bp
Eight base recognition site occurs 4⁸ (65,536) bp

Average fragment size (bp) produced by different enzymes

Enzyme	Target	Arabidopsis	E. Coli	Human
<i>Apal</i>	GGGCCC	25000	15000	2000
<i>BamHI</i>	GGATCC	6000	5000	5000
<i>SpeI</i>	ACTAGT	8000	60000	10000

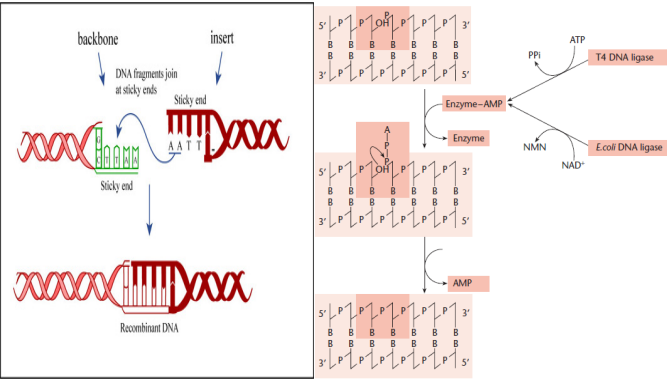
Lecture No. 39
Cutting DNA molecules

Joining DNA molecules

Methods of joining DNA fragments : Mainly three methods are used for joining DNA in vitro i). Joining covalently annealed cohesive ends by DNA ligase ii). Joining blunt-ended fragments by DNA ligase from phage T4 infected *E. coli*

Lecture No. 42
Joining DNA molecules

iii). The third utilizes the enzyme terminal deoxynucleotidyltransferase to synthesize homopolymeric 3' single stranded tails at the ends of fragments
DNA ligase: *E. coli* and phage T4 encode enzyme, DNA ligase, which seals single stranded nicks between adjacent nucleotides in a duplex DNA chain
DNA ligase to create covalent recombinant DNA: DNA ligase can repair the nicks produced after the association of cohesive ends of DNA strands
Use of DNA ligase to create a covalent DNA recombinant.



Summary of restriction endonucleases: Type II restriction enzymes are heavily responsible for the current explosion in the field of gene manipulation in that they are essential in forming recombinant DNA. .

Lecture No. 40

Joining DNA molecules-- DNA modifying enzymes

Nucleases, DNA Polymerase, Reverse transcriptases, DNA ligases

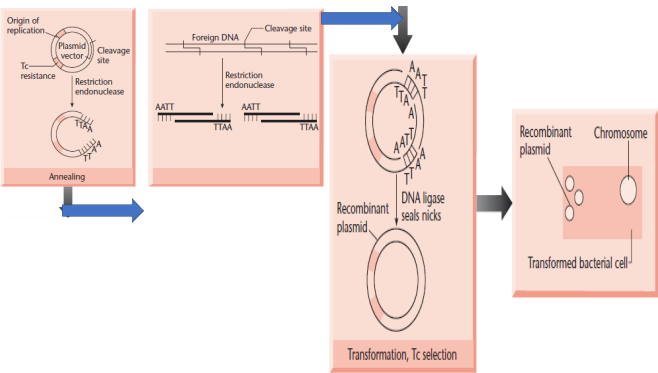
Nucleases: Nucleases or DNases are the enzymes that degrade DNA. Two broad classes of nucleases i). Exonucleases ii). Endonucleases

DNA Polymerase: An enzyme that catalyzes template-dependent synthesis of DNA

Reverse transcriptases: An RNA-directed DNA polymerase in retroviruses; capable of making DNA complementary to an RNA

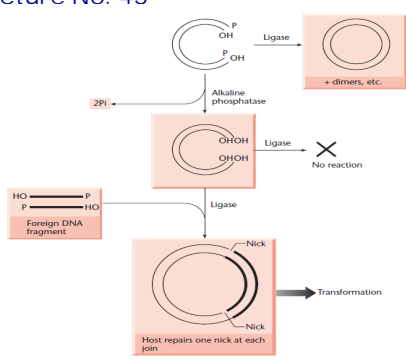
DNA ligase: An enzyme that creates a phosphodiester bond between the 3' end of one DNA segment and the 5' of another

Lecture No. 41



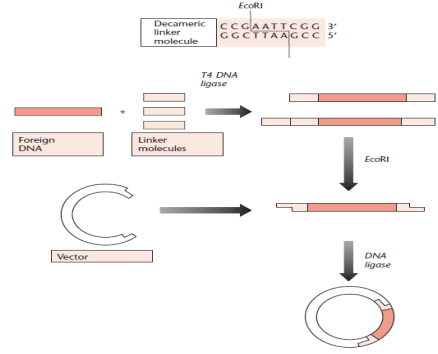
Lecture No. 43

Joining DNA molecules
Alkaline Phosphatase: An enzyme responsible for removing phosphate groups from many types of molecules including DNA. Treatment of linearized plasmid vector DNA with alkaline phosphatase to remove 5'-terminal phosphate groups will prevent recircularization and plasmid dimer formation. Application of alkaline phosphatase



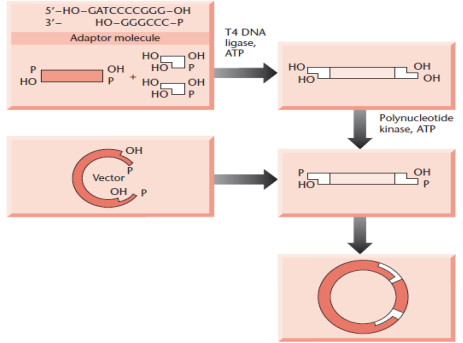
Lecture No. 44

Joining DNA molecules
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.



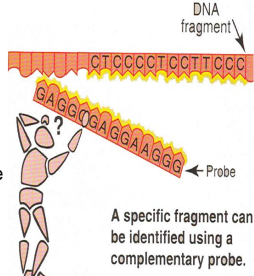
Lecture No. 45

Adaptors: Chemically synthesized adaptor molecules which have a preformed cohesive end can be used to insert blunt ended foreign DNA into vector. The main difference between linker and adaptor is that former having blunt ends while later have one cohesive end.

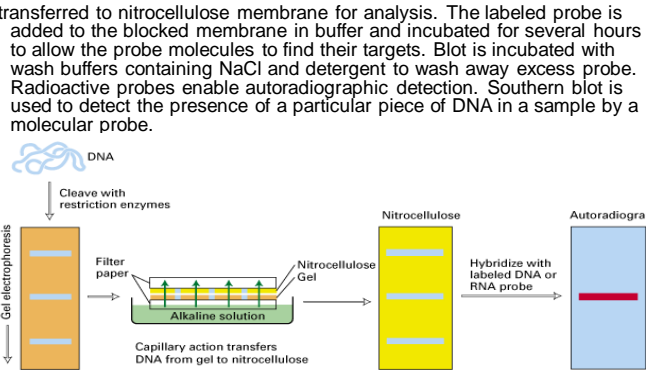


Lecture No. 48

SOUTHERN BLOTTING PROBES –PROBES: Labeled material to detect a target. For DNA: 20-30 nucleotides, complementary to a region in the gene or DNA.
RADIOACTIVE PROBE - P32: Sensitive Relatively cheap Hazardous Radioactive waste disposal regulations should be followed
NON-RADIOACTIVE PROBE –BIOTIN: Sensitive Relatively expensive..
HYBRIDIZATION OF PROBES: The binding between single stranded labeled probe to a complementary nucleotide sequence on the target DNA..
PROBES: Labeled material to detect complementary region in the gene or DNA.



Lecture No. 46
SOUTHERN BLOTTING: Southern Blotting is named after its inventor, the British Biologist Edwin Southern (1975).
BLOTTING: Southern Blotting is named after its inventor, the British Biologist Edwin Southern (1975).
OTHER BLOTTING METHODS: Other blotting methods with similar principles, but using protein or RNA, have been named in reference to Edwin Southern's name after.
SOUTHERN BLOTTING
Identify DNA sequence (gene) of interest.
Identified DNA may be a small piece of DNA or a mutation
Lecture No. 47
SOUTHERN BLOTTING 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



Lecture No. 49
TRANSFER METHODS IN SOUTHERN BLOTTING
TRANSFER METHODS OF DNA TO MEMBRANE
UPWARD TRANSFER OF DNA TO MEMBRANE

Upward capillary transfer, Downward capillary transfer, Simultaneous transfer to two membranes, Electrophoretic transfer, Vacuum transfer.



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 is used in many ways while studying RNAs.

Lecture No. 53

WESTERN BLOTTING : A technique used to detect the presence of a specific protein in a complex protein mixture. 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 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 . A technique used to detect the presence of a specific protein in a complex protein mixture.

Lecture No. 54

Lecture No. 50
MUTATIONS IDENTIFICATION BY SOUTHERN BLOTTING

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. 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). Estimation of the size & number of the bands generated after digestion of the genomic DNA will be different. Mutations can be identified by Southern blotting

Lecture No. 51
NORTHERN BLOTTING

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

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

Lecture No. 52

NORTHERN BLOT 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.

WESTERN BLOTTING PROCEDURE

PROCEDURE

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

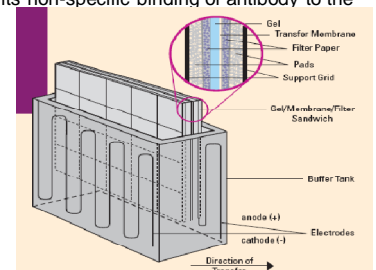
PROCEDURE: 1- The choice of extraction method depends primarily on the sample and whether the analysis is targeting all the proteins in a cell or only a component from a particular subcellular fraction.

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.

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.

ELECTROPHORETIC TRANSFER



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

WESTERN BLOTTING

A molecular technique to study proteins.

By
Vu MediCal zone-
biotechnologists

Remember us in your
prayers

