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**DEPARTMENT OF MICROBIOLOGY**

**QUESTION BANK**

**Title of the paper: Recombinant DNA Technology**

**Subject Code:16SMBEMB2**

**2MARKS**

**UNIT-I MILESTONES OF RDNA**

**RDNA:**

Joining together of DNA molecules from two different species that are inserted into a host that to produce a new genetic combination

**VALUE OF RDNA:**

1. Medicine
2. Agriculture
3. Industry

S**.COHEN & H.BOYER**

In 1973, discovered recombinant plasmid PSC101

**GENETIC ENGINEERING**

The manipulation of genetic make up of living cells by inserting a desired gene through a DNA vector is called **Genetic engineering**. This method is otherwise called as **Biomolecular engineering.**It was discovered by **Stanley Cohen & Herbert Boyer in 1973.**

**RECOMBINANT DNA/CHIMERIC DNA/CHIMERAS:**

Gene is a small piece of DNA i.e encodes a specific protein

↓

This gene is inserted into vector DNA

↓

The DNA formed by joining DNA segments of two different organisms

↓

Recombinant DNA/Chimeric DNA formed

**r-DNA technology**

The gene is introduced into a cell in the form of recombinant DNA. The gene manipulation is called as r-DNA technology.

**RECOMBINANT / GENETICALLY MANIPULATED ORGANISM:**

* The organism where genetic make up is manipulated using r-DNA technique is called recombinant (or) genetically manipulated organism(GMO)

**METHODS IN GENETIC ENGINEERING:**

The various steps involved in genetic engineering are:

1. Preparation of desired genes
2. Isolation of DNA vector
3. Construction of recombinant DNA
4. Induction of r-DNA into host cell
5. Selection & multiplication of r-DNA
6. Expression of cloned gene

**PREPARATION OF DESIRED GENE:**

Genomic DNA

↓

Cut into several pieces by using restriction enzyme

↓

The pieces of DNA are separated on the basis of lengths by electrophoresis

↓

DNA segments of desired length used for cloning

**PREPARATION OF DESIRED GENE (another method):**

mRNA

 ↓Reverse Transcriptase

Desired gene

↓

Complementary DNA/cDNA

↓

Used for gene cloning

**PREPARATION OF DESIRED GENE (another method):**

Desired gene

↓

Synthesised

↓

Computerised machine

↓

DNA synthesized/Gene machine

**11.DONOR DNA**

If the number of copies DNA is not enough for cloning, the gene(cDNA) is multiplied by PCR (Polymerase chain reaction). The desired DNA is otherwise called as Passenger DNA/Target DNA/Donor DNA/Insert DNA

**ISOLATION OF DNA VECTOR:**

* The DNA used to transfer the desired DNA into a host cell / organism is known as Vector
* The other name for vector is Cloning vector, Carrier molecule, Cloning vehicle,Vehicle.
* Two types of Cloning vectors
1. Plasmids
2. DNA of Virus (˨)

**CONSTRUCTION OF RECOMBINANT DNA:**

The desired DNA is inserted into vector DNA using restriction enzyme DNA ligase

VECTOR DNA + DESIRED DNA

↓

RECOMBINANT DNA/r-DNA/CHIMERA /CHIMERIC DNA/

RECOMBINANT VECTOR

**RECOMBINANT**

* The recombinant DNA were allowed to multiplied inside the cell.
* The selected cell containing r-DNA is called as **recombinant (or) Cloned gene (or) Transformant**

**APPLICATION OF GENETIC ENGINEERING IN AGRUCLTURE:**

* Insect resistant tomato plant
* Virus resistant tobacco & Potato
* Petunia with attractive flowers
* Transgenic cattle for more milk
* Transgenic sheep & goat with high lactatin
* Transgenic chicken for more eggs & better meat
* Transgenic fish with high growth rate

**APPLICATION OF GENETIC ENGINEERING IN HEALTH**

* Human insulin in bacteria
* vaccines for cholera, typhoid, small pox
* Interferons for viral diaseases
* Monoclonal antibodies to treat diseases

**APPLICATION OF GENETIC ENGINEERING IN INDUSTRY**

* Hepatitis B Vaccine
* Foot & mouth disease vaccine
* Antibodies Production
* Steroid hormone production

**3 STEPS OF DNA EXTRACTION**

1. Lysis of Chromosome & Cell membrane
2. DNA purification by Protein and cell membrane
3. Protein of DNA by buffer

**HOMOGENIZATION:**

* Growth medium on the cells was discarded and cells washed with ice-cold 1XPBS solution
* then cells were mixed with trisazol
* The cells were lysed and homogenized by repeated pipetting

**c-DNA LIBRARY**

Complementary DNA (cDNA) is a double stranded complement of mRNA

Protein

↓

m-RNA

 ↓Reverse Transcriptase

C-DNA

* C-DNA is synthesized from m-RNA by Reverese Transcriptase techniques
* The cDNA are cloned into a cloning vector plasmid for creating Cdna library
* Developed by Okayama & Berg (1982)

**GENOMIC LIBRARY**

* Genomic library is the collection of all the genes of an organism
* It is also called as gene bank
* The genomic library of an organism is obtained by a technique called shot gun cloning

**STEPS FOLLOWED GENOMIC LIBRARY:**

1. Isolation of DNA fragment of a cell
2. Isolation of Vectors
3. Insertion of DNA fragments into vectors
4. Introduction of r-DNA into host cells
5. Cloning the host cell
6. Identification of cloned genes
7. Use of clones for the construction of genomic library

**UNIT-II DNA ENZYMES**

**TAILORING ENZYMES**

Some purified enzymes are used to cut and join DNAs. The enzymes useful for rDNAS are known as tailoring enzymes (or) Tools of genetic engineering.

**THE IMPORTANT DNA TAILORING ENZYMES ARE**

1. Reverse Transcriptase
2. Ribonuclease-H
3. S1Endonuclease
4. Klenow enzyme
5. Taq DNA Polymerase
6. Restriction Endonuclease
7. Terminal nucleotidyl transferase
8. Alkaline phosphatase
9. Polynucleotide kinase
10. DNA Ligase

**REVERSE TRANSCRIPTASE:**

* Reverse transcriptase is an enzyme that makes a DNa strand using mRNA as a template strand
* It is also known as RNA dependent DNA synthetase
* It is isolated from Retroviruse (eg: Rous Sarcoma Virus **RSV**, Mouse Mammary Tumour Virus **MMTV**, Avian Myeloblastosis Virus **AMV**, and Murine Leukemia Virus **MuLV**)
* Reverse transcriptase is used to make CDNAs from mRNAs.

**RIBONUCLEASE-H (RNAse-H):**

* Ribonuclease-H is a nuclease enzyme that selectively hydrolyses the mRNA present in a RNA-DNA hybrid
* It is isolated from retroviruses such as RSV,AMV, MMTV, MuLV, etc.,
* It is found associated with reverse transcriptase enzyme
* The molecular weight of this enzyme is 92000 daltons
* RNAse-H is used to hydrolyse the mRNA present in RNA-DNA hybrid for releasing the cDNA to synthesize the second strand
* It is used to reisolate DNA Probe from an RNA-DNA hybrid, if the RNA is no need.

**KLENOW ENZYME (OR) KLENOW FRAGMENT:**

* Klenow enzyme is a product of enzymatic breakdown of DNA Polymerase I from E.coli
* The molecular weight of this enzyme is 76000 daltons
* It has 5’ – 3’ polymerase activity and has 3’ – 5’ exonuclease activity
* It has the ability to do DNA polymerization under invitro condition
* It is also used in PCR for the amplification of DNA

**S1 NUCLEASE:**

* S1nuclease is an enzyme that selectively cuts and degrades single-stranded portions of DNA.
* It is a glycoprotein consisting of 82% protein and 18% carbohydrates units
* The molecular weight of the enzyme is 38000 daltons
* This enzyme breaks the phosphodiester bond between two nucleotides in single stranded portion of DNA and then degrades single stranded portions of DNA and RNA
* S1 nuclease is used to degrade the hair pin loop formed

**Taq DNA POLYMERASE:**

* Taq DNA Polymerase is isolated from the gram negative, rod shaped, thermophilic bacterium *Thermus aquaticus*
* It consists of a single polynucleotide chain having the molecular weight of 95,000 daltons
* It is a thermostable enzyme that can withstand heat at 95®C or more.
* This enzyme is resistant to PH 9
* Taq DNA Polymerase is used to make numerous identical copis of a desired DNA segment by polymerase chain reaction.

**RESTRICTION ENDONUCLEASES:**

* The nuclease enzyme that cuts the DNA at a unique sequence is called restriction endonuclease
* They cut the DNA in a non-terminal region.
* Restriction endonucleases are used to generate rejoinable DNA fragments
* They are also known as molecular knives, molecular scissors, restriction enzymes (or) molecular scalpels
* The sequence recognized by the restriction enzyme to cut the DNA is called restriction site, restriction endonuclease site (or) recognition site. The recognition site consist of 4-8 base pairs

**TYPES OF RESTRICTION ENZYME:**

The restriction endonucleases are grouped into three types. They are:

1. Type I restriction endonucleases
2. Type II restriction endonucleases
3. Type III restriction endonucleases
* Eg:ECoRI, Hind III, etc.,

**TYPE II RESTRICTION ENDONUCLEASE:**

* A type II restriction endonuclease recognize a specific sequence in the duplex DNA and cuts the DNA at the recognized sequence.
* So the cutting is SQUENCE SPECIFIC. The enzyme consists of two identical sub-units and its molecular weight ranges from 20000-100000 daltons
* It requires Mg2+ as cofactor for the enzyme activity.
* At present 350 type II restriction endonucleases are isolated from various bacterial strains.

**PLANE OF CUTTING:**

* Some restriction enzymes cut DNAs along the axis of symmetry of the restriction sites. They break down two phosphodiester bonds, one in either strand of the restriction site , at the axis of symmetry.
* Several restriction enzymes cut one strand at left side of the axis of symmetry and the other strand at the right side of the axis.
* As a result, DNA fragments with single-stranded extensions are formed. The single stranded extensions are called **cohesive ends** (or) **sticky ends.**

**ISOCHIZOMERS:**

* Restriction enzymes of different organisms , that recognize the identical sequence to cut the DNA, are called Isoschizomers.
* They belong to type II restriction endonuclease
* These enymes consist of two identical subunits and they are 20000-100000 daltons in molecular weight
* They require Mg2+ as cofactor for enzyme activity

**TERMINALNUCLEOTIDYL TRANSFERASE:**

* Terminal nucleotidyl transferase is an enzyme that adds mononuclotide triphosphates to 3”OH group of DNA fragments.
* While adding the nucleotide, a pyrophosphate is released free
* This enzyme adds nucleotides without athe aid of a template strand. It is made up of two non-identical subunits and the molecular weight is 32000 daltons
* It is used to make radioactive DNA probes.

**ALKALINE PHOSPHATASE:**

* Alkaline phosphatase digests the terminal phosphate group at the 5” end of DNA fragment. It acts on both the DNA and the RNA
* The enzyme consists of two identical subunits and has the molecular weight of 1,40,000 daltons.
* There are four zinc atoms in an alkaline phosphatase molecule.
* Alkaline Phosphatase is used to remove the 5’ phosphatase group from a linearised vector DNA

**POLYNUCLEOTIDE KINASE:**

* Polynucleotide kinase transfers a phosphate from ATP to 5’-OH group of dephosphorylated DNA (or) RNA. It consist of four identical sub-units and its molecular weight is 34,000 daltons.
* It is used to transfer radioactive P32 from ATP to dephosphorylated 5’ end of r-DNA (or) RNA for labeling.
* The labeling technique is used:
1. to make hybridization probes
2. to make diagnostic kits
3. to analyse the base sequence of DNA
4. to construct restriction map.

**DNA LIGASE:**

* DNA ligase is an enzyme that joins the ends of two duplex DNAs to make a long DNA. This process is called Ligation.
* It seals the nick by establishing a covalent bond between 5’ phosphate group and 3’ OH group at the nick.
* The bond is called Phosphodiester bond. This enzyme never seals the nick, if there is no 5’ phosphate group (or) if one (or) more nucleotides are missing.
* DNA ligase isolated from E.Coli requires ATP amd NAD +  for enzyme activity. However, DNA ligase of Lambda T4 phage requires ATP alone to catalyse the ligation.
* This enzyme is called as T4 DNA Ligase. It is 68,000 daltons in molecular weight.
* DNA ligase is used to join a vector DNA and a target DNA to construct recombinant DNA.
* It is used to join DNA fragment of different organisms for making vectors with desired characters.
* It is used to add linker and adaptor sequences to blunt ended vector DNA and target DNA.

**METHYLASES (OR) METHYL TRANSFERASES:**

* Methylase is an enzyme that adds a methyl group to cysteine and adenine of DNA. It is a monomeric enzyme.
* The molecular weight of methylase is 62000 daltons.
* Methylase adds a methyl group to N-6 position of adenine to 6-methyl adenine and N-5 position of cysteine to form 5-methyl cytosine
* It adds only one methyl group at a time and immediately dissociate from the DNA. To add a second methyl group, the enzyme once again bind to the DNA.
* The methylated base in a DNA is shown by an astrich mark (\*) near the base.

**UNIT-III CLONING VECTORS**

**GENE CLONING VECTORS**

* The DNA that carries the desired gene to the host cell is called gene cloning vector. It is also known as cloning vector, vector, cloning vehicle, carrier DNA.
* Plasmids, Viral DNAs , Cosmids are used as gene cloning vectors

**PLASMIDS:**

* Plasmids are circular, double-stranded DNAs usually present in prokaryotic cells. They can carry a foreign DNA of 5-15 Kbp size to bacteria. Eg:pBR322.
* Some plasmids carry genes to plant cells. Eg: Ti Plasmid.

**VIRAL DNA:**

* They are linear or circular and single stranded or double stranded DNAs.
* DNAs of ɻ phage and M13 phage are used to carry genes to bacteria.
* DNA of simian virus 40 (SV40) is used to carry genes to animal cells.
* Viral DNAs can carry DNAs of 10-25 kbp size.

**COSMIDS**

 They are a type of constructed plasmids containing complementary single stranded sites (cos-sites) of ɺ DNA. They can carry DNA segments of 25-45 kbp size of bacteria.

 Eg: pHV79

**SIMPLE STEPS OF rDNA**

A suitable cloning vector is chosen

↓

Cut with Restriction Enzyme

↓

Desired gene is inserted into cleaved vector using DNA Ligase

↓

The resulting recombinant vector(r DNA) is then introduced into a host cell for gene manipulation.

**PLASMIDS**

* Plasmids are small, circular, double-stranded, extra-chromosomal DNAs present in bacterial cells.
* They are inherited sharply without the help of chromosomal DNA.
* They replicate due to independently due to the presence of an origin of replication.
* They are 1kbp- 200 kbp in size.

**COPY NUMBER**

* They have limited number of genes. Most bacteria contain more than one copy of each plasmid. The number of copies present in a cell is called **COPY NUMBER**.
* The copy number of plasmids usually varies from 1 to 50
* This copy number is further increased by treating with Chloramphenicol.

**EPISOMES**

* Some plasmids, under certain conditions, integrated into the chromosomal DNA of the bacterium. Such plasmids are called as EPISOMES.
* The intergrated plasmid replicate along with the chromosomal plasmid. Eg:F-Plasmid
* The eukaryotes except yeast do not have plasmids

**YEAST PLASMID:**

The yeast Saccharomyces cerevisiae contain

YEp...Yeast Episomal Plasmid/2-micron plasmid

YIp.... Yeast Integrating Plasmid

ARS-Automatically replicating sequence

**CLASSIFICATION OF PLASMIDS BASED ON TRANSFER**

1. CONJUGATIVE PLASMID
2. NON-CONJUGATIVE PLASMID

**CONJUGATIVE PLASMID**

* These plasmids transferred from one bacterium to another bacterium. They contain tra genes.
* Eg:F-Plasmid

**NON-CONJUGATIVE PLASMID**

* These plasmids do not pass from one bacterium to another bacterium
* Eg: Col E1 Plasmid

**CLASSIFICATION OF PLASMIDS BASED ON FUNCTIONS**

* 1. F-PLASMID
	2. R-Plasmid
	3. Col-Plasmid
	4. Degradative Plasmid
	5. Virulence Plasmids

**F-PLASMID**

* Fertility plasmid
* Contain some genes expressing the maleness in bacteria
* the genes are known as tra gene
* Eg:F-plasmid

**R-Plasmid**

* Resistance Plasmid
* It contain genes giving resistance to bacteria against antibiotics and heavy metals
* Eg:pSC101-gives the organism the organism tetracycline resistance

**Col-Plasmid**

* Codes for the synthesis of bacterial toxin Colicin
* it kills other closely related strains of the bacteria
* Eg:ColE1, Col B

**Degradative Plasmid**

* Codes for enzymes that degrade toxic substances –toluene,xylene,parathion, etc
* TOL plasmid of Pseudomonas putida involves the break down of toluene.

**Virulence Plasmids**

* Provide pathogenecity to bacteria

**ISOLATION & PURIFICATION OF PLASMIDS**

Mixture of Plasmids

 ↓ Ethidium Bromide (⁺)

Ultracentrifugation in Cscl2 density gradience

↓

.Plasmids form a specific layer

↓

Storage in TE Buffer

**PLASMID AS CLONING VECTORS**

* **A.Chang & N.Cohen (1973)** Ist proved the use of plasmids as cloning vectors.
* They isolated plasmids from 2 different strains of bacteria and fused them using **restriction enzyme and DNA ligase.**
* The fused DNA **(Chimeric plasmid)** was then introduced into E.coli cells & its expression was carried out.

**GENETIC MARKERS**

* The plasmid must have 1 or few genetic markers.
* These markers help us for the selection of organism that has rDNA

**ORIGIN OF REPLICATION**

* The plasmids must have its own origin of replication and regulatory genes for self replication.

**UNIQUE RESTRICTION SITES**

* The plasmids have unique restriction sites for commmon restiction enzymes in use

**INSERTIONAL INACTIVATION**

* The plasmids must have unique sites for RE in marker genes. This will help us for the selection of recombinants

**NATURAL PLASMIDS**

* Some plasmids are isolated from bacteria and directly used for gene cloning without any modification.
* Eg: RP4 plasmid of Pseudomonas, Col E1 of E.coli, YEp & YIp of yeasts
* Most of the plasmids cannot be used for gene cloning.

**This is because.......**

* They are larger in size
* They have no genetic markers
* They have no unique site for common restriction enzymes in the marker gene
* They confer pathogenecity to the host

**BASED/ ARTIFICIAL PLASMIDS**

* In order to overcome the above difficulties, some unwanted portions are cut out from the wild type plasmid and desired sequences are inserted in it.
* The effective cloning vectors created from wild type plasmids are called **based plasmid vectors, artificial vectors, derived vectors, constructed vectors.**
* Eg:pBR322, RSF1010, pSC101

**PBR322**

* It is an artificial plasmid
* It is a gene cloning vector for E.coli
* It was constructed from two plasmids---Psc101 & ColE1, transposan Tn3
* In the plasmid,,,,
* **P**-it is a plasmid
* **BR**- the name of the workers F.Bolivar & Rodriguez who created the plasmid
* **322-**specific no.to distinguish the plasmid from others

**PBR322**

* pBR322 is a circular, double stranded plasmid DNA
* It consist of 4363 basepairs
* It has 55 restriction sites for 66 restriction enzymes
* It has 2 selectable marker genes
* Tetr-Tetracycline resistance gene
* Ampr-Amphicillin resistance gene



**PBR322 –ADVANTAGES:**

* It is a small plasmid contain 4363 basepairs
* Copy number is 15. the copy number is increased by 3000 by adding chloramphenicol to the bacterial culture.
* Bacterial cell uptake DNA of 15kbp size. But pBR322 is only 4.4kbp in size, we can used it as vectors
* The regulation and expression of a gene inserted into the plasmid is good.
* It has been used as a base plasmid for constructing pUC8, Puc9,pUCV10

**USES OF PBR322 :**

* It is being used to introduce desired genes into E.coli cells
* Eg:somatostatin gene of man is introduced into E.coli through pBR322

**PUC8**

* It is a cloning vector constructed from plasmid pBR322
* It is 2676 bp in size
* It has **Amphicillin resistance gene** (Ampr )and **origin of replication** (ori) from pBR322, **Lac Z gene** of E.coli.
* The Lac Z gene has a multiple cloning sequence (MCS)
* Foreign gene is inserted into MCS and it inactivates the Lac Z’ gene
* The recombinants were screened by growing the cell in a medium containing X-gal
* Eg: pUC7, pUC9, pUC12,pUC13,pUC18,pUC1

**COSMIDS**

* COSMID---*It is an artificial plasmid containing* cos-sites *of ɺ DNA.*
* It is formed by joining ends of a linearised plasmid DNA with cos-sites of a ɺ DNA.
* It is a derived vector
* It is linear inside the phage capsid.
* It get circularised and behave like plasmid

**DEFINITION FOR COSMIDS**

* It is a circular, DS DNA
* It has 2 SS regions at both ends of a plasmid DNA.
* It do not code for phage proteins and phage lysis
* It has origin of replication from plasmid DNA
* It has selectable marker genes and gene cloning sites of plasmid DNA
* It exist as extrachromosomal DNA and multiply independently
* EX:Cosmid pLFR5, Cosmid Pjb8, Cosmid pHC79

**Cosmid pLFR5**

* It is commonly used cloning vector
* 6Kbp in size
* Constructed from E.coli plasmid pBR322 & 2 cos-ends of ɺ DNA
* It has origin of replication (ori), Tetr gene.
* It has MCS-Multiple cloning site b/n ori & cos site

**Cosmid pJB8**

* It is commonly used cloning vector
* 5.4 Kbp in size
* Constructed from E.coli plasmid pBR322 & 2 cos-ends of ɺ DNA
* It has origin of replication (ori), Ampr gene.
* It has MCS-Multiple cloning site b/n ori & cos site

**Cosmid pHC79**

* It is commonly used cloning vector
* 6.5 Kbp in size
* Constructed from E.coli plasmid pBR322 & 2 cos-ends of ɺ DNA
* It has origin of replication (ori), two marker genes -Ampr & Tetr gene.
* It has MCS-Multiple cloning site b/n ori & cos site

**ADVANTAGES OF COSMIDS**

* It pick up large number of DNA fragments than plasmids
* As it pick large number of DNA, it is used to construct gene library
* It help to study the sequence of DNA of organism
* It help to clone large genes in bacteria

**PHAGEMIDS**

* A phagemid is a hybrid vector that has origin of replication from a plasmid and a ɺ phage DNA.
* It is constructed by inserting a linearised plasmid DNA into a cleaved ɺ DNA. this process is known as **lifting the plasmid**
* Phagemids help for invivo multiplication of phage particles
* Eg: ɺ ZAP

**YEAST ARTIFICIAL CHROMOSOME (YAC)**

* It is a derived cloning vectors used to clone large DNA fragments in yeast cells.
* They are linear in shape
* Each YAC is made up of 3 important regions
1. **Telomeres**
2. **Centromere**
3. **Autonomously replicating sequence (ARS)**
* The YAC has all three sequences is called a minichromosome
* It behaves like an additional chromosome in yeast cell---Eg:YAC2

**USES OF YAC:**

* YAC is used to form g**enomic libraries** of prokaryotes and eukaryotes
* It is used in human genome project (HGP) to construct **gene map of chromosomes** in man
* It is used to clone large DNA fragments for **gene walking**, a method for screening gene library

**UNIT IV GENE TRANSFER METHODS**

**DNA TRANSFER METHOD**

The foreign DNA is directly introduced into Plant genome by Physical /Chemical methods is called DNA transfer methods.

**TYPES OF DIRECT DNA TRANSFER METHODS:**

1. Physical gene transfer method
2. Chemical gene transfer method

**PHYSICAL GENE TRANSFER METHOD**

1. ELECTROPORATION
2. BIOLISTIC
3. MICROINJECTION
4. LIPOSOME FUSION

**CHEMICAL GENE TRANSFER METHOD**

1. POLYETHYLENE GLYCOL (PEG)
2. DEAE

**ELECTROPORATION:**

It involves the use of high foeld strength electrical impulses to reversibly permeabilize the cell membranes for the uptake of DNA.

This technique can be used for the delivery of DNA into plant cells and protoplast.

**BIOLISTICS (PARTICLE BOMBARDMENT METHOD)**

* **BIOLISITICS=BIOLOGICAL + LISTICS**
* **OTHER NAME= PARTICLE GUN**

**GENE GUN**

**BIOBLASTER**

* Used for DNA transfer in mammalian cells and microorganisms
* Discovered by **Stanford** (1988)

**Microcarriers**

Microcarriers (Microprojectiles) the tungsten (or) gold particles coated with DNA are copied by microcarriers (Macroprojectiles)

↓

Microcarriers are inserted into apparatus

↓

Pushed downward by rupturing the disc

↓

Stopping plate does not permit the movement of macrocarrier

↓

microcarrier will moved propelled at an high speed into plant material

↓

DNA segment are released and integrated with the genome

**MICROINJECTION:**

It is a direct method involving the mechanical insertion of the desired DNA into a target cell.

The technique for the transfer of gene through a micropipette (0.5-10µm) into cytoplasm/ nucleus of a plant cell.

↓

While the gene transfer is clone, the receipient cells are kept immobilized in agarose and a hole is made up of by a suction holding pipette

↓

Transformed cell is cultured and grown into transgenic plant

**POLYETHYLENE GLYCOL-MEDIATED TRANSFORMATION (PEG)**

Isolation of Protoplast suspension

↓

Addition of Plasmid DNA

↓

40% PEG (dissolved in mannitol +calcium nitrate solution)

↓

Incubate 24 hours

↓

Protoplast transformed

**CALCIUM MEDIATED CO-PRECIPITATION MEDIATED TRANSFER**

DNA mix with Cacl2 + PO4 buffer

↓

DNA-CaPO4 precipitate

↓

Keep for several hours

↓

Cells get transformed

↓
Addition of DMSO increase the efficiency of transformation

**SELECTION & SCREENING OF RECOMBINANTS**

 After the introduction of rDNA into suitable host cells, it is essential to identifiy those cels which have received the rDNA molecules . This process is called Screening (or) Selection.

**SCREENING/SELECTION- DIRECT METHOD**

1. Selection by Complementation
2. Marker inactivation methods

**SCREENING/SELECTION- INDIRECT METHOD**

1. Immunological method
2. Genetic method

**INSERTIONAL INACTIVATION METHOD:**

* This is more efficient than the direct selection
* Antibiotics resistance genes act as a good insertion inactivation system
* Plasmid PBR322 contain 2 antibiotic genes
* one for ampr gene
* one for tetr gene

**BLUE-WHITE SELECTION METHOD**

* Another powerful method of screening for the presence of recombinant plasmid is called as blue-white selection
* This method is based upon the insertional inactivation of the Lac-Z gene present on the vector Eg:PUC19
* LacZ gene express the enzyme β-galactosidase whose activity can cleave a colorless substrate called X-gal into a blue cloured product

**ANTIBIOTIC SENSITIVITY ASSAY**

Muellor Hintor agar medium

↓

Prepared

↓

Sterilized

↓

Swab the microorganisms on the surface of the medium

↓

Place the antibiotics disc on the media surface

Eg:Amphicillin disc

Tetracycline disc

Chloramphenicol disc

 ↓

Incubate at 24 hours for 37®C

↓

Zone of inhibition was seen as clear zones

**COLONY HYBRIDIZATION:**

* It is the method of selecting bacterial colonies with desired genes
* This method was discovered by **Grunstein & Hogness.**

Bacterial colony grown on nutrient agar

↓

Replicate on nitrocellulose filter paper (direct contact)

↓

Cells lysed

↓

DNA denatured

↓

Add radiolabelled DNA/RNA

↓

Autoradiography

**UNIT – V BLOTTING**

**EASTERN BLOTTING**

* It is given by Bogdanov
* This method is used to identify carbohydrates epitopes including glycoconjugates and lipids.
* Blotted proteins were analysed after transferring into membrane are analysed by using probes.

**POLYMERASE CHAIN REACTION (PCR)**

* **Polymerase chain reaction** (**PCR**) is a common laboratory technique used to make many copies (millions or billions!) of a particular region of DNA.
* The polymerase chain reaction is a laboratory technique for generating large quantities of a specified DNA. It was developed by Karry mullis in 1984.PCR is now considered as a basic tool for the molecular biologist.

**PRINCIPLE OF PCR:**

* Three steps were followed in PCR
1. DENATURATION
2. RENATURATION (OR) ANNEALING
3. SYNTHESIS

These three steps denaturation, renaturation and synthesis are repeated again and again to generate multiple forms of target DNA.

**ESSENTIALS OF PCR:**

* The essential requirement for PCR are listed below:
	+ - * A target DNA (100-35000 bps) in length
			* Two Primers that are complementary to regions flanking the target DNA
			* Four deoxyribonucleotides
			* A DNA Polymerase that can withstand at a temperature upto 95®C

**RENATURATION (OR) ANNEALING:**

* As the temperature of the mixture is slowly cooled to about 55®C, the primers base pair with the complementing regions flanking the target DNA strands.
* This process is called renaturation (or) annealing.**SYNTHESIS:**
* The initiation of DNA synthesis occurs at 3’ hydroxyl end of each primer. The primers are extended by joining the bases complementary to DNA strands.
* The temperature has to be kept optimal as required by the enzymes DNA polymerase.
* For Taq DNA Polymerase, the optimum temperature is around 75®C

**BIOCHIP/DNA CHIP/DNA MICROARRAYS**

A computer chip made by inserting a semiconducting molecule into a protein frame work on a protein support is known as biochip.Biochips replace the usual silicon chips in modern computers.Protein molecules in the framework have their own characteristic three dimensional structure.

**DNA CHIPS (MICROARRAYS):**

DNA chips (or) DNA microarray are recent development for DNA sequencing.A large number of probes , each one with different sequence are immobilized at defined positions on the solid surface made up of either nylon (or) glass.The probes can be short DNA molecules such as cDNA (or) synthetic oligo nucleotides.

**APPLICATION OF DNA CHIPS :**

* Identification of genes responsible for the development of nervous system
* Detection of genes responsible for inflammatory diseases
* Prokaryotes has been identified.

**BLOTTING:**

Blotting is a technique in which nucleic acid i.e RNA and Dna or proteins are transferred onto a specific membrane. The membrane may be nitrocellulose , PVDF or nylon membrane.

**SOUTHERN BLOTTING:**

Southern blotting is a laboratory technique used to detect a specific DNA sequence in a blood or tissue. A restriction enzyme is used to cut a sample of DNA into fragments that are separated using gel electrophoresis. The DNA fragments are transferred out of the gel into membrane and read by autoradiography.

**WESTERN BLOTTING:**

Western blotting is otherwise called as protein immunoblotting is an analytical technique used to identify and locate specific proteins in a sample of tissue homogenate or extract based on their ability to bind to specific antibodies.

**RAPD:**

RAPD stands for Random Amplification of Polymorphic DNA . It is a type of PCR, but the segment of DNA that are amplified are. Random. RAPD creates short primers, then proceeds with PCR using a large template of genomic DNA and so the DNA fragments are amplified.

**RFLP:**

RFLP stands for Restriction fragment length polymorphism. It is a technique that exploits variations in homologous DNA sequence, known as polymorphism, in order to distinguish individual populations.

**DNA FINGER PRINTING:**

Dna finger printing is a method used to identify an individual from a sample of DNA by looking at unique patterns in their DNA.I is a laboratory technique used for identify the parentage, gnes, genetically character and hereditary characters.

**DNA AMPLIFICATION:**

DNA amplification is the production of multiple copies of a sequence of DNA. Repeated copying of a piece of DNA. DNA amplification plays a role in cancer cell. A tumour cell amplifies or copies DNA fragments as a result of cell signals and sometimes environment events.

**ANSWER THE FOLLOWING (5 MARKS)**

**UNIT I MILESTONES OF RDNA TECHNOLOGY**

1. Short notes on RDNA technology
2. Applications of RDNA technology
3. Mention the features of genetic engineering
4. Write about genetically manipulated organisms
5. Write about the preparation of desired gene
6. Mention the applications of genetic engineering
7. Comment on the isolation of DNA
8. Comment on the purification of DNA
9. Write about the isolation of RNA
10. Mention the purification of RNA
11. Describe the cDNA library
12. Describe the genomic library
13. Difference between cDNA and genomic library

**UNIT –II DNA ENZYMES**

1. Short notes on DNA modifying enzymes
2. Write about DNA tailoring enzymes
3. Salient features of Genetic engineering tools
4. Write about the type of DNA enzymes
5. Mention the features of Restriction enzymes.
6. Short notes on Restriction endonuclease
7. Types of restriction endonuclease.
8. Importance of DNA ligase in genetic engineering
9. Write about the important features of methylase

**UNIT-III CLONING VECTORS**

1. Explain about the gene cloning vectors
2. Structure of plasmid
3. Character of plasmid
4. Steps used for the construction of rDNA
5. Classification of plasmid based on transfer
6. Classification of plasmid based on functions
7. Isolation of plasmid
8. Purification of plasmid
9. Features of natural plasmid
10. Features of artificial plasmid
11. Short notes on PBR322
12. Write about hybrid vectors
13. Short notes on cosmids
14. Notes on yeast artificial chromosome
15. Notes on bacterial artificial chromosome

**UNIT-IV GENE TRANSFER METHODS**

1. Write about the DNA transfer methods
2. Short notes on physical gene transfer method
3. Short notes on chemical gene transfer method
4. Mention the steps in biolistic method
5. Write about the microinjection method
6. Salient features of antibiotic sensitivity assay
7. Write about the blue-white selection method
8. Explain the colony hybridization method
9. Write about replica plating
10. Short notes on microcarriers

**UNIT-V BLOTTING**

1. Write about theblotting techniques
2. Describe the eastern blotting
3. Comment on western blotting
4. Mention the notes on northern blotting
5. Salient features of PCR
6. Write about the steps about PCR
7. Short notes on DNA finger printing
8. Write about the applications of DNA finger printing
9. Mention the importance of DNA chips
10. Write about the DNA microarrays
11. Write about biochips
12. Mention the application of recombinant DNA technology.
13. Difference between RFLP & RAPD
14. Write about RFLP.

**EXPLAIN BRIEFLY (10 MARKS)**

**UNIT I MILESTONES IN RDNA TECHNOLOGY**

1. Explain the isolation and purification of DNA in detail.
2. Explain the isolation and purification of RNA in detail
3. Explain about the steps adopted for genetic engineering in a detailed manner
4. Describe about the construction of rDNA in detail
5. Explain the history of recombinant DNA technology .

**UNIT-II DNA ENZYMES**

1. Explain in detail about the DNA modifying enzymes along with its uses
2. Explain about the types of enzymes used as tools in genetic engineering.
3. Explain about the discovery of restriction endonucleases and its types in a detailed manner.
4. Explain the application of DNA tailoring enzymes in detail

**UNIT-III CLONING VECTORS**

1. Explain the gene cloning vectors in detail.
2. Explain the isolation and purification of plasmids in a brief manner
3. Mention the classification of plasmids in detail
4. Explain about the plasmids and cosmids in detail.

**UNIT-IV CLONING VECTORS**

1. Explain about the DNA transfer methods in detail
2. Explain about the physical gene transfer methods in a brief manner
3. Explain about the chemical gene transfer methods in a detailed manner
4. Explain about the selection and screening methods of recombinants
5. Explain the immunological methods in detail
6. Explain the genetic methods adopted for the gene transfer methods indetail.

**UNIT-V BLOTTING VECTORS**

1. Explain the blotting techniques in detail
2. Explain the southern blotting in detail.
3. Describe the steps followed for PCR and add notes on its types.
4. Describe the steps followed for DNA finger printing in detail.
5. Describe the DNA microarrays in detail.
6. Explain about the RAPD in a detailed manner.
7. Explain the RFLP and its applications