Sem : IV

Paper IV: Plant Molecular Biology and Genetic Engineering

I. Choose the correct answer

1. Restriction enzyme Bam HI is a
(A) Phosphatase
(B) Exonuclease
(C) Endonuclease
(D) Both b and c

2. Southern hybridization is process to detect
(A) DNA
(B) RNA
(C) Protein
(D) All of the above

3. Reverse Transcription PCR is used to
(A) RNA strand is reverse transcribed into its complementary DNA
(B) amplify DNA sequence
(C) quantify the copy number of nucleotide sequences.
(D) All of the above.

4. Which method is used for screening the libraries based on gene expression?
(A) Colony hybridization
(B) Plaque hybridization
(C) Functional complementation
(D) PCR screening

5. Which of the screening method uses antibody sandwich?
(A) Colony hybridization
(B) PCR screening
(C) Functional complementation
(D) Immunological screening

6. Which of the following method is called as Chain elongation method?
(A) TA cloning
(B) Gateway cloning
(C) Plaque Hybridization
(D) Sanger Sequencing

7. Particle bombardment method is also termed as
(A) Particle gun,
(B) Micro projectile bombardment
(C) Biolistic
(D) All of the above

8. Combining multiple gene into a single genotype is called
(A) Marker Assisted Selection
(B) Gene Pyramiding
(C) Genetic Mapping
(D) Molecular mapping

9. Molecular Markers include
(A) RFLP
(B) RAPD
(C) AFLP
(D) All of these

10. Which of these is a false statement?
(A) Markers must be tightly-linked to target loci.
(B) Markers must be more than 5 cM.
(C) Using a pair of flanking markers greatly improves the reliability.
(D)The markers should not be polymorphic.

11. How do we confirm the presence of foreign gene in transformed plants?
(A) Western Blot
(B) Northern Blot
(C) Southern Blot
(D) All of the above

12. Which of the following is a reporter gene?
(A) Kanamycin resistance gene
(B) Hygromycin phosphotransferase gene
(C) β-glucuronidase (GUS)
(D) Xgluc

II. Fill in the Blanks

1. Restriction endonucleases are named according to the organism in which they were ----- using a system of -------and ----------.

2. The restriction map allows researchers to correlate the genetic map and the physical map of a chromosome.

3. The restriction mapping of DNA which involves the size analysis of restriction fragments and nucleotide sequencing of DNA

4. The tandem repeats of short sequences are called mini satellites or variable number tandem repeats (VNTRs), such repeats are used as genetic markers in personal identity.

5. The chemical method of sequencing developed by Maxam and Gilbert

6. The enzymic method of sequencing is Sanger's or Dideoxynucelotide Chain Terminators

7. A genomic library is an organism specific collection of DNA covering the _____ of an organism.

8. Adapters are short stretches of oligonucleotide with _____ or a linker digested with _____ prior to ligation.

9. The two classical methods of sequencing are _____ and _____ sequencing.

10. Co-integrate vectors are the _____ derivatives of Ti-plasmids.

11. Plastid genomes resemble _____ genomes in many aspects and also contain some features of _____ organisms, such as RNA editing and split genes.

12. RAPD provides predominantly --------- markers.

What are the basic steps involved in polymerase chain reaction (PCR)?
Ans. The steps involved in basic polymerase chain reaction (PCR) are as follows **:**
a) Initial Denaturation- Denaturation of the template double stranded DNA to single strands at a temperature of 94–96°C for 7-10 minutes.

b) Denaturation- Heating the reaction mixture to 94–98°C for 20–30 seconds. It results in melting of the double stranded DNA by disrupting the hydrogen bonds between complementary bases, yielding single-stranded DNA molecules.
c) Annealing- Following denaturation, the primers are allowed to anneal to the single-stranded DNA templates at 50–65°C for 40–60 seconds. Typically the annealing temperature should be about 3-5 °C below the T m of the primers.
d) Extension- Extension/elongation step includes addition of dNTPs to the 3' end of primer with the help of DNA polymerase enzyme at a certain temp of 68-72°C for 50sec-1minute.
The above three steps (Denaturation, annealing and extension) are repeated for 30-35 cycles.
e) Final extension- This step is performed for 5–15 minutes at a temperature of 70–74°C after the last PCR cycle to ensure amplification of any remaining single-stranded DNA.
Final hold step at 4°C may be done for short-term storage of the reaction mixture.

Write the functions of Genetic Engineering Approval Committee (GEAC).
Ans: Genetic Engineering Approval Committee (GEAC) functions under the Department of Environment (DOEn) as statutory body. It reviews and approves various activities involving large scale use of genetically engineered organisms and their products in research and development, industrial production, environmental release and field applications.
The functions include giving approval from environmental view on:
i. Import, export, transport, manufacture, process, selling of any microorganisms or genetically engineered substances or cells including food stuffs and additives that contains products derived by gene therapy.
ii. Discharge of genetically engineered/classified organisms/cells from laboratory, hospitals and related areas into environment.
iii. Large scale use of genetically engineered organisms/classified microorganisms in industrial production and applications.
iv. Deliberate release of genetically engineered organisms.

Describe the basic steps in Northern Blot reaction.
Ans. The northern blotting involves the following steps:
a) Total RNA is extracted from a homogenized tissue sample or cells. Further eukaryotic mRNA can then be isolated by using of oligo (dT) cellulose chromatography to isolate only those RNAs by making use of a poly A tail.
b) The isolated RNA is then separated by gel electrophoresis.
c) The RNA samples separated on the basis of size are transferred to a nylon membrane employing a capillary or vacuum based system for blotting.

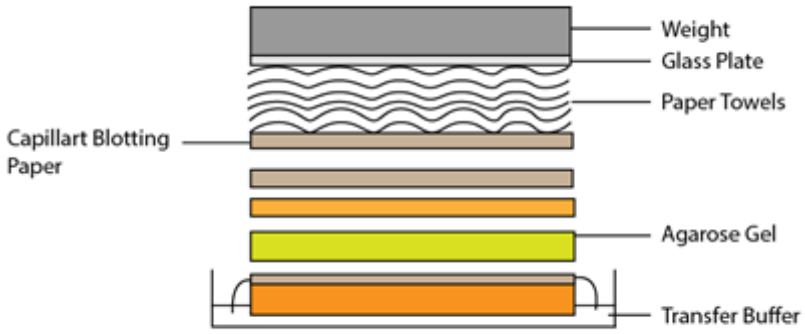

Fig: Setup for Northern blotting
d) Similar to Southern blotting, the membrane filter is revealed to a labeled DNA probe that is complementary to the gene of interest and binds.
e) The labeled filter is then subjected to autoradiography for detection.

Describe the plaque lift method of screening of libraries?
Ans. Plaque lift, also known as Plaque hybridization employs a filter lift method applied to phage plaques and was developed by Benton and Davis in 1977. This is a widely used method successfully employed to isolate the recombinant phage by nucleic acid hybridization. The method of screening library by plaque hybridization is described below-
- The nitrocellulose filter is placed on the upper surface of master agar plates, making a direct contact between plaques and filter.

- The plaques contain phage particles, as well as a considerable amount of unpackaged recombinant DNA which bind to the filter.
- The DNA is denatured, fixed to the filter, hybridized with radioactive probes and assayed by autoradiography.

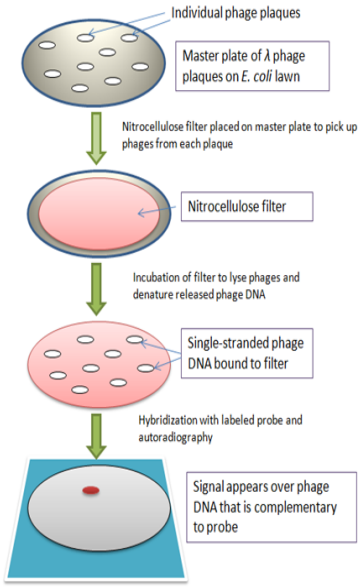

Fig: Schematic representation of Plaque hybridization screening method.

What are the various components of Ti-Plasmid?
Ans. Ti-plasmid has following components described as below

1. T- DNA

It is a small, specific segment of the plasmid, about 24kb in size and found integrated in the plant nuclear DNA at random site. This DNA segment is flanked by right and left borders (25 bp each). Deletion of the RB repeat abolishes T-DNA transfer, but the LB seems to be non-essential. The LB repeat has little transfer activity alone.

Genes on T-DNA

The T-DNA contains two groups of genes, which possess the ability to express in plants as follows-

- **Oncogenes** for synthesis of auxins and cytokinins (phytohormones). The over-production of phytohormones leads to proliferation of callus or tumour formation.
- **Opine synthesizing genes** for the synthesis of opines (a product from amino acids and sugars secreted by the crown gall infected cells and utilized by *A. tumefaciens* as carbon and nitrogen sources). Thus opines act as source of nutrient for bacterial growth, e.g. Octopine, Nopaline.

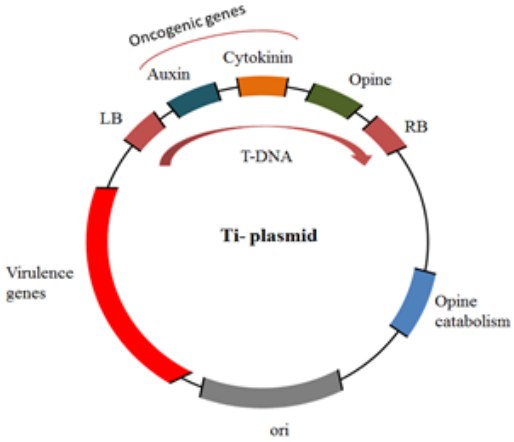

Fig: Ti-plasmid of Agrobacterium

2. Virulence genes (vir genes)

They aid in the transfer of T-DNA into the host plant cell. There are 35 *vir* genes arranged in 8 operons in a Ti plasmid.

Ri plasmid

Agrobacterium rhizogenes is a soil born gram negative bacterium. It causes hairy root disease of many dicotyledonous plants. The ability of *A. rhizogenes* to incite hairy root disease is confirmed by a virulence plasmid, which is similar to that found in *Agrobacterium tumefaciens* which causes Crown gall tumors of plants. The virulence plasmid of *A. rhizogenes* is commonly known as the Ri-plasmid (pRi). The pRi have extensive functional homology with the pTi. The pRi contains distinct segment(s) of DNA, which is transferred to plant genome during infection. The transfer T-DNA to the plant genome is mediated by another segment on the plasmid known as the virulence (vir) region. All strains of *A. rhizogenes* are known to produce agrocinopine.

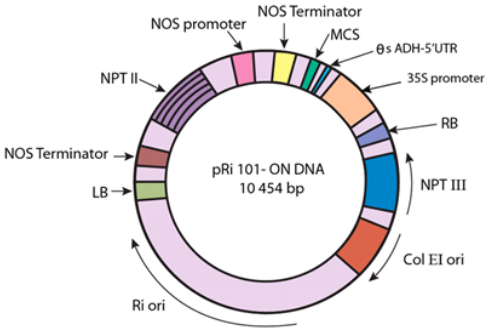

Figure 26.2: Ri plasmid