3. Biotechnology - Process and Application

3.0 : Introduction

Q.1. Define the term 'Biotechnology'.

Aris: Biotechnology is a branch of biology which deals with the techniques of using live organisms, enzymes or biological processes to produce products and provide services for human welfare.

Q.2. What are the different applications of biotechnology ?

Ans: i) Biotechnology is multidisciplinary and involves cell and molecular biology, micro biology, genetics, biochemistry, physiology, etc.

ii) It has been used since ages in activities such as brewing, wine-making, bread-making, food preservation, modification by fermentation (e.g. milk products and vinegar), etc.

iii) It is also used in the manufacture of soaps, drugs, dyes and fertilizers.

iv) During the past 2-3 decades, biotechnology has brought total revolution, advancement and superiority in various fields.

v) Genetic engineering has made it possible to map the whole genome of an organism, to carry out gene transfers, gene cloning, etc. Recombinant DNA technology has helped to develop growth hormones, interferons, vaccines against viral and malarial diseases, etc.

vi) Biotechnology has increased the industrial production ofa1cohol, enzymes, beverages, antibiotics, vitamins, hormones, organic acids, etc.

vii) Some important areas of biotechnology are Microbial Biotechnology, Agricultural Biotechnology, Animal Biotechnology, Forensic Biotechnology, Bioremediation, Aquatic Biotechnology and Medical Biotechnology.

viii) In-vitro fertilization leading to a test tube baby, synthesizing a gene, correcting a defective gene are all possible due to biotechnology.

Q.3. Enlist the fields in which biotechnology is used.

Ans: Biotechnology has wide applications in the field of agriculture, medicine, chemical industry, pharmaceutical industry and environment.

Q.4. Define Modern biotechnology.

Ans: i) Modem biotechnology is the integration of natural science and organisms, cells, parts and molecular analogues for products and services.

ii) This definition is given by The European Federation of Biotechnology (EFB).

3.1 : Recombinant DNA technology

Q.5. Define the terms :

i) Recombinant DNA

ii) Vector

iii) Transformation

v) Transfection

v) Transduction

Ans:i) **Recombinant DNA :** The chimeric DNA formed by the insertion of fragment having the desired gene into a cloning vector is called Recombinant DNA.

ii) **Vector :** Vector is a DNA molecule used to transfer genetic material into another cell.

iii) **Transformation:** Insertion of vector in a bacterial cell is called transformation.

iv) **Transfection:** Insertion of vector in a eukaryotic cell is called transfection.

v) **Transduction: Insertion of a viral vector is called as transduction.**

Q.6. What are 'Sticky ends' ?

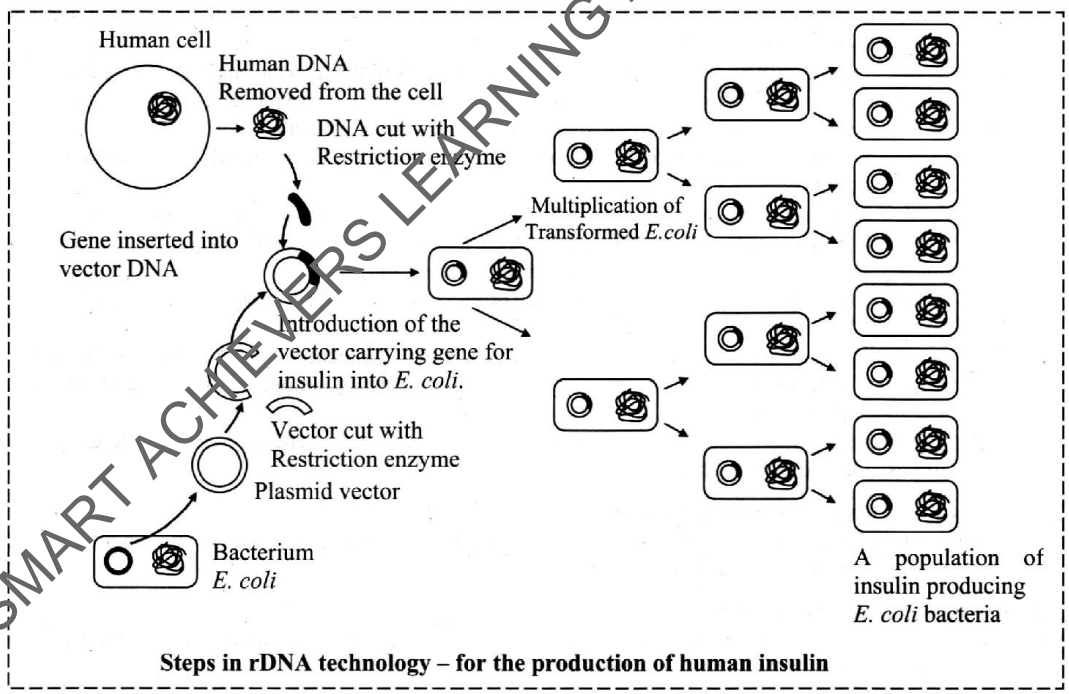
Ans: The short single stranded extensions (unpaired nucleotides) produced at the end of a cleaved DNA that can base pair with complementary sticky ends of another DNA cut with the same enzyme are called 'Sticky ends'

Q.7. Explain the term Chimeric DNA.

Ans: The vector DNA containing a newly introduced desired gene is called chimeric DNA. It is also called as recombinant DNA.

Q.8.Sketch and label the process showing steps in recombinant DNA technology for cloning of human insulin gene in E.coli.

Ans:



Steps in rDNA technology – for the production of human insulin

Q.9. Give examples of the therapeutic products made by recombinant DNA technique.

Ans :

No.	Therapeutic product	Examples
i)	Blood proteins	Erythropoietin; Factors VII, VIII, IX, Tissue plasminogen activator, Urokinase
ii)	Human hormones	Epidermal growth factor, Follicle Stimulating hormone, Insulin, Nerve growth factor, Relaxin, Somatotropin
iii)	Immune modulators	α – Interferon, β – interferon, Colony stimulating factor, Lysozyme, Tumor necrosis factor
iv)	Vaccines	Cytomegalovirus, Hepatitis B, Measles, Rabies.

Q.10. What is recombinant DNA technology ?

Ans: i) The addition, removal, replacement or repair of a part of genetic material resulting into the desirable change of the phenotype of an organism is called recombinant DNA technology.

ii) It is also called'gene manipulation' or 'genetic engineering' or 'gene splicing', etc.

iii) All the activities in recombinant DNA technology are done very carefully under highly controlled laboratory conditions.

iv) The field of recombinant DNA technology has vast scope and tremendous variety of applications.

Q.11.Describe the important biological tools used in recombinant DNA technology.

Ans: The biological tools used in recombinant DNA technology are as follows:

i) Enzymes : It requires use of different enzymes such as :

No.	Class of enzyme	Examples
a.	Lysing enzyme	Lysozyme
b.	Cleaving enzymes (Molecular scissors)	Restriction endonucleases
c.	Synthesizing enzymes	Reverse transcriptase; DNA polymerase
d.	Joining enzyme	DNA ligase
e.	Manipulating enzyme	Alkaline phosphatase

ii) Vector : It is a carrier DNA molecule to which the fragments of desired DNA are attached and carried to the required site. It may be a plasmid or cosmid DNA or bacteriophage DNA or DNA from plant and animal viruses or artificial DNA, etc.

iii) Target DNA or Foreign DNA or Desired Gene : The desired fragments of DNA (gene or genes) which are to be transferred from one organism to the other by using some vector is called target DNA. e.g. Nif gene, insulin gene, etc. The cell or the organism from which the desired gene is taken is called 'donor'.

iv) Host : It is the cell where recombinant DNA is allowed to multiply to produce several copies. e.g. Bacteria, yeast, etc. The host should be non-pathogenic, harmless microorganism which is easy for cultivation. The bacterium Escherichia coli is the most commonly used host in recombinant DNA technology.

Q.12. Describe the steps in rDNA technology.

Ans: The following steps are involved in genetic engineering or r-DNA technology :

i) Isolation of desired gene :
The donor individual having desired gene is selected.
From the DNA of this donor, desired gene is selected and isolated with the help of restriction endonuclease enzyme.
The donor DNA containing the desired gene is called passenger DNA.

ii) Selection of vector :
A vector DNA (usually plasmid DNA or Phage DNA) is selected.

iii) Formation of recombinant DNA :
The vector DNA is cleaved at a specific point using restriction endonuclease enzyme. The cut ends of vector DNA are sticky, i.e. cohesive. The desired gene is now ligated with the vector DNA using ligase enzyme. The vector DNA containing a new introduced gene is called recombinant DNA (r-DNA) or chimeric DNA (Chimeric vector or chimeric plasmid).

iv) Gene transfer to the host :
This chimeric plasmid is introduced into bacterial cell (Host cell). e.g. *E.coli* for cloning. Bacillus *subtilis* and *Saccharomyces cerevisiae* can also be used as host. Such a bacterial cell with chimeric or r-DNA is called transformed host. The incorporation of r-DNA into bacterial cell is generally done by electroporation.

v) Cloning :
The transformed bacterial cell is now allowed to grow on the nutrient medium where it multiplies rapidly. It results in the formation of a large number of transformed bacterial cells. All these cells have a copy of recombinant or chimeric DNA. Generally, after introduction ofr-DNA or chimeric plasmid in the host cell, amplification is done. It is a process in which the number ofr-DNA in a bacterial cell is increased.

Q.13.Describe the common characteristics of vector.

Ans: The common characteristics of vector are :

i) The cloning vector must be able to replicate in the host cell.
ii) It must have an origin of replication.
iii) Cloning vector should be amplified in the host cell.
iv) Large amount of the desired foreign DNA should be produced inside the cloning vector.
v) Cloning vector should have restriction endonuclease recognition sites.
vi) Cloning vector should also have a marker gene which will facilitate the identification and selection of transformants and elimination of non-transformants.

Q.14.Name some vectors commonly used in rDNA technology.

Ans: Some of the common vectors for bacteria are plasmids, cosmids, lambda phage, Baculovirus (useful in insects), Ti plasmid (useful for plants) and YAC (Yeast Artificial Chromosome useful for yeast cells).

Q.15.Can you suggest a method to remove oil (hydrocarbon) from seeds based on your understanding of rDNA technology and chemistry of oil ?

Ans:i) Oils are a group of lipids, which are unsaturated and in liquid form at room temperature.

ii) Lipids are broken down or digested by enzymes called lipases.

iii) The genes encoding lipases can be introduced into the seeds (plants that produce seeds) which may digest such lipids.

3.2 Transposons, Plasmids, Bacteriophages

Q.3. 16. Define the terms :

i) Plasmid

ii) Clone

Ans:i) Plasmid: Plasmids are small, extrachromosomal, double stranded, circular forms of DNA that replicate autonomously.

ii) Clone : A clone is a cell, cell product or organism that is genetically identical to the unit or individual from which it was derived.

Q.17. Give an account of transposons.

Ans.i) **Transposons** are the sequence of DNA that can move around to different positions within the genome of a single cell.

ii) Transposons are also called **"Jumping genes"** and are examples of mobile genetic elements.

iii) This process of transposons to move from one position to another on a chromosome is called **transposition.**

iv) Transposons can jump from chromosomal DNA to plasmid DNA and back.

v) In humans, some sequences of interspersed- repetitive DNA are present. This common form of transposons are called 'Alu'sequence.

vi) There are two types of trans po sons - retrotransposons and DNA transposons.

vii) Due to their ability of insertion into the genome, transposons can cause mutation with variety of effects.

viii) They may turn nearby genes off, preventing their ability to create a protein or they may turn them on, thus increasing the amount of protein made.

Q.18.What are 'jumping genes' ? **[Mar 2014]**

Ans: The sequences of DNA that can move or transpose themselves to new positions within the genome of a single cell are known as 'jumping genes'.

Q.19. Distinguish between Retrotransposons and DNA transposons.

Ans:

No.	Retrotransposons	DNA transposons
i)	They involve RNA intermediate	They do not involve RNA intermediate
ii)	Retrotransposons function with Reverse transcriptase enzyme	Transposons function with the transposase enzyme
iii)	Retrotransposons copy in two stages, DNA $\xrightarrow{\text{Transcription}}$ RNA RNA $\xrightarrow{\text{Reverse Transcription}}$ DNA	The enzyme transposase makes a staggered cut at the target site producing sticky ends. Then, the transpososns are cut out and ligated in new position.
iv)	They use copy and paste mechanism	They use cut and paste mechanism

Q.20.Explain how plasmids are used as vectors in genetic engineering.

Ans:i) Plasmids are the most widely used cloning vectors in genetic engineering. The gene to be replicated is inserted into copies of a plasmid containing genes that make cell resistant to particular antibiotics.

iii) Then, the plasmids are inserted into bacteria by the process of transformation.

iv) Bacteria are then exposed to a particular antibiotic which facilitates identification and selection of transformants, because only bacteria that take up copies of the plasmid survive, since the plasmid makes them resistant.

v) Now, such bacteria can grow in large amounts, harvested and lysed to obtain the required plasmid.

vi) By this process, plasmids are also used to make large amount of proteins from the inserted gene. e.g. insulin.

Q.21.What are plasmids? Which plasmids are widely used in genetic engineering ?

Ans: Plasmids are the most widely used cloning vectors in genetic engineering. Plasmids are circular, double stranded, extra-chromosomal, self replicating DNA molecules. pBR 322 is one of the earliest plasmid vector constructed and most widely used in genetic engineering. It contains two different antibiotic resistance genes such as ampicillin resistance and tetracycline resistance, also recognition sites for a number of restriction enzymes. Other plasmid vectors are pUC, shuttle vectors. The pBR 322 and pUC vectors can replicate only inside. bacterial cells, while shuttle vectors are those vectors which can exist in *E. coli* as well as in eukaryotic cells.

Q.22. What are bacteriophages? Describe how they are used as vectors.

Ans: Bacteriophages are viruses which infect the bacterial cell. Bacteriophages are widely used as cloning vector in genetic engineering as they have the ability to replicate in the host cell.

Most widely used bacteriophages are lambda (λ) phage, M13 phage.

Lambda (λ) phage:

i) It has a double stranded linear DNA with 48.5 kb in length, of which twelve bases at each end are unpaired but are complementary. These sticky or cohesive ends are called the cos sites (cohesive-end sites) of 12 bp.

ii) A large portion in the central region of the genome of A phage is not necessary for the lytic cycle of the virus in E.coli cells. So, vectors have been designed in such a way that this region is substituted by a foreign DNA.

iii) They allow cloning of DNA fragments upto 23 kb in size.

Use of bacteriophage as a vector :

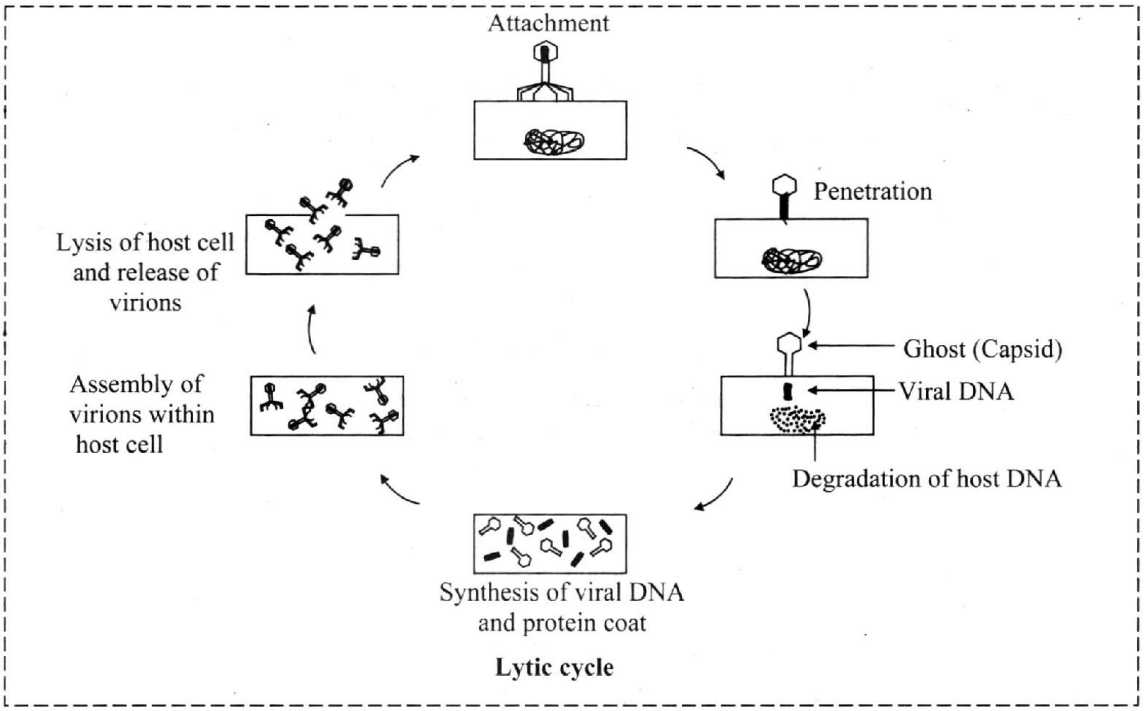
Bacteriophage infects bacterial cells by injecting their DNA into the bacterial cell. The viral DNA injected into the cell replicates and expresses itself in the host cell resulting in the formation of a number of phages which lyse the bacterial cell and come out. This property of bacteriophage to transfer its DNA into specific bacterial host cells during infection is used in genetic engineering.

Q.23.Give brief account of replication of bacteriophages.

Ans:Replication of bacteriophages, (lytic cycle) inside the specific hostlbacterial cell takes place in the following steps :

i) Attachment : Bacteriophages attach to specific receptors on the surface of bacteria. As phages do not move independently, they rely on random encounters with the right receptors.

ii) Penetration : After attachment, the tail fibres bring the base plate closer to the surface of the cell. Once attached completely, the tail contracts, injecting genetic material (DNA) through the bacterial membrane. (Capsid - protein coat remains outside and is called 'ghost')



Lytic cycle

iii) **Synthesis of proteins and nucleic acid :** The host's normal synthesis of proteins and nucleic acids is disrupted, and it is forced to manufacture viral DNA, and proteins instead. These products are the parts of new virions within the cell or proteins involved in cell lysis.

iv) **Virion assembly :** The base plates are assembled with the tails first. The head (capsids) are constructed separately and then are joined with the tails. The DNA is packed efficiently within the head. The whole process takes about 15 minutes.

v) **Release of virions:** Phages are released via. lysis of cell. It is achieved by an enzyme called endolysin, which breaks down the cell wall. Released virions are capable of infecting a new bacterium.

3.3 : Restriction Fragments

Q.3. Q.24. Who isolated restriction endonucleases for the first time ?

Ans: Steward Linn and Werner Arber isolated two enzymes in 1963 which restricted the growth of bacteriophage in bacterium *E. coli.*

Q.25. What are the two types of nucleases? What is the difference between their function?

Ans: i) Two types of nucleases are exonucleases and endonucleases.

ii) Exonucleases are the enzymes which remove nucleotides from the ends of the DNA.

iii) Endonucleases are the enzymes which make cuts at specific positions within the DNA.

Q.26. Write about the nomenclature of restriction enzymes.

Ans: i) Restriction endonucleases are named by a standard procedure, with particular reference to the bacteria from which they are isolated.

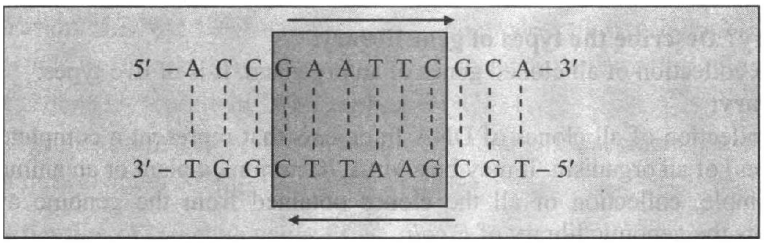
ii) The first letter (in italics) of the enzymes indicates the genus name followed by the first two letters (also in italics) of the species, then comes the strain of the organism and finally a Roman numeral indicating the order of discovery.

iii) For example: **EcoR I** is from Escherichia (E) coli (co) strain Ry 13 (R) and first endonuclease (I) to be discovered.

Hind III is from Haemophilus (H) influenzae (in) strain Rd (d) and the third endonuclease (III) to be discovered.

Q.27.What are palindromes? Give example.

Ans: Palindrome in DNA is a sequence of base pairs that reads the same on the two strands when orientation of reading is kept the same. Following sequence is an example of palindrome :



Q.28. What are restriction fragments? Explain with respect to restriction enzymes.

Ans: Restriction fragment is a DNA fragment resulting from the cutting of a DNA strand by a restriction enzyme (restriction endonucleases).

The particular sequence of nucleotides on DNA where a restriction enzyme cuts the two strands of DNA is called recognition site.

Most recognition sites are **palindromic** (the sequence of nucleotides is the same on both strands when read in the 5' to 3' direction) and are four to eight nucleotides long.

A particular DNA molecule will always yield the same set of restriction fragments when exposed to the same restriction enzyme.

Restriction fragments produced by restriction enzymes have sticky ends which are significantly used for producing recombinant DNA.

Q.29. Which procedure is employed to separate the DNA fragments formed by restriction enzymes?
Ans: Gel electrophoresis is employed to separate the DNA fragments formed by restriction enzymes.

Q.30. Describe briefly the Origin of replication.
Ans: i) Origin of replication is a sequence on DNA from which replication starts.
ii) Any piece of alien DNA linked to this sequence ca~ be made to replicate within the host cells.
iii) This sequence is also responsible for controlling the copy number of the linked DNA.
iv) In prokaryotic DNA, there is a single origin of replication, while the giant eukaryotic DNA has several origins of replication.

Q.31. Write a note on restriction enzymes.
Ans: Restriction enzymes belong to the class of enzymes, called nucleases. They are of three kinds:
i) Exonucleases ii. Endonucleases iii. Restriction endonucleases
i) Exonucleases: These are a group of restriction enzymes, which remove the nucleotides from the 5' or 3' ends of a single stranded DNA or at only a single strand of a double stranded DNA.
iii) Endonucleases: These are the enzymes, which cut the double stranded DNA at any point along the length except the ends, but of only one strand.
iii) Restriction Endonucleases. These enzymes cut both the strands' of DNA at specific sites on both strands of DNA. There are three major types of restriction endonucleases, type I, type II and type III, but only type II is useful in genetic engineering because they can be used in-vitro to recognise and cut at specific DNA sequences.

3.4 : Preparing and cloning a DNA Library

Q.3.32. What a~e the two types of gene library?
Ans: There are two types of gene library :
i) Genomic library
ii) cDNA library.

Q.33. What is gene library? Describe the types of gene library.
Ans: Gene library: It is a collection of all cloned genes of an organism. It is of two types :
i) Genomic library :
a) It is a collection of all clones of DNA fragments that represent a complete set of genes (DNA sequences) of an organism. It may be a virus, bacterium, a plant or an animal.
b) For example, collection of all the clones obtained from the genome of E.coli collectively represents the genomic library of Ecoli.
c) For constructing a genomic library of an organism, its entire genome (DNA) is isolated and cut into fragments of equal size by restriction endonuclease enzyme.
d) These fragments are .then inserted into cloning vectors.
e) The recombinant vectors are then transferred to a suitable organism such as bacteria, yeast or a virus
f) Each organism contains one fragment. These transformed organisms are cultured to produce their clones and are stored.
ii) cDNA library :
a) The library constructed by using cDNA is called cDNA library.
b) cDNA is a copy of DNA (complementary DNA) produced by using m-RNA in the process of reverse transcription.
c) Enzyme reverse transcriptase helps in this process.
d) This process is also called 'Terninism', since Temin and Baltimore discovered it.
e) For construction of cDNA library, cDNA is inserted into a suitable vector like a phage or plasmid and then cloned in a proper host. e.g. E.coli.

Q.34. Write a note on cDNA library.
Ans: i) The cDNA library represents the DNA of only eukaryotes and not prokaryotes.
ii) The cDNA library can be constructed by using mRNA because mRNA are highly processed, intron free representatives of DNA having only coding sequence.
iii) cDNA fragment is prepared directly by using mRNA as template using different biochemical methods.
iv) Since cDNA clone is prepared by using a specific mRNA, there is no need of screening the

cDNA clones.

v) The cDNA clone with desired function may be few or large in number.

vi) Therefore, all these clones must be ligated (joined) to a suitable vector and transferred into the host bacteria.

vii) Each bacterial cell possess a single DNA clone, hence collection of all recombinant bacteria is called cDNA library. The bacteria are maintained on maintainance media for several years.

Q.35.Explain the term D A probe.

Ans: DNA probe: DNA probe is a small DNA segment with known nitrogen bases that recognise complementary sequence in a DNA molecule and thus allows identification and isolation of specific D A sequence from an organism.

Q.36. What are the applications of cDNA library ?

Ans:i) Eukaryotic cDNA can express itself in bacterial cell. Production of human proteins such as interferons, insulin, blood clotting factor VIII can be done by using bacterial cultures having cDNA.

ii) Single stranded cDNA with radioactive labelling is very useful in genetic engineering.

3.5 : Gene Amplification (PCR)

Q.37. Explain the terms :

i. Gene amplification

ii. Denaturation'

iii. Annealing

Ans:i) Gene amplification: Gene amplification is the process of obtaining multiple copies of known DNA sequence containing the desirable gene in a relatively short time.

ii) Denaturation of DNA: DNA denaturation is the process by which double-stranded deoxyribonucleic acid unwinds and makes single stranded DNA through the breaking of hydrogen bonds between the bases. It is done by heating of DNA at about 91°C.

iii) Annealing: The pairing of primers to single stranded DNA segment is called annealing. This requires a temperature at about 55°C.

Q.38. Explain amplification of the gene using peR.

Ans:i) Amplification of the genelD A segment refers to the process of making multiple copies of the gene. Selective amplification is carried out by Polymerase Chain Reaction (PCR).

ii) The basic principle of this technique is that when a double stranded DNA molecule is heated at a high temperature, the two strands of DNA separate to produce two single stranded DNA molecules.

iii) If these two single stranded molecules are used as templates and copied with the help of a polymerase enzyme, two molecules of the original DNA type are formed.

iv) If these events are repeated a number of times, multiple copies of the DNA segments can be generated, i.e. the original DNA fragment/molecule is amplified.

Q.39.Define PCR.

Ans:PCR (Polymerase Chain Reaction) is an in vitro technique of gene amplification used to generate billions of copies of a particular DNA sequence in a short time.

Q.40. What are the requirements for PCR ?

Ans: The basic requirements for PCR include:

i) DNA fragment to be amplified (100-35,000 bp in length).

ii) Two primers, which are oligonucleotides of about 17-30 nucleotides.

iii) Four types ofdeoxyribonucleotides, dNTPs such as dATP, dCTP, dGTP, dTTP.

iv) DNA polymerase, which is stable at a high temperature (above 94°C) used during the separation of the two strands. The commonly used thermostable polymerase is Taq polymerase, that is isolated from the bacterium, *Thermus aquaticus.*

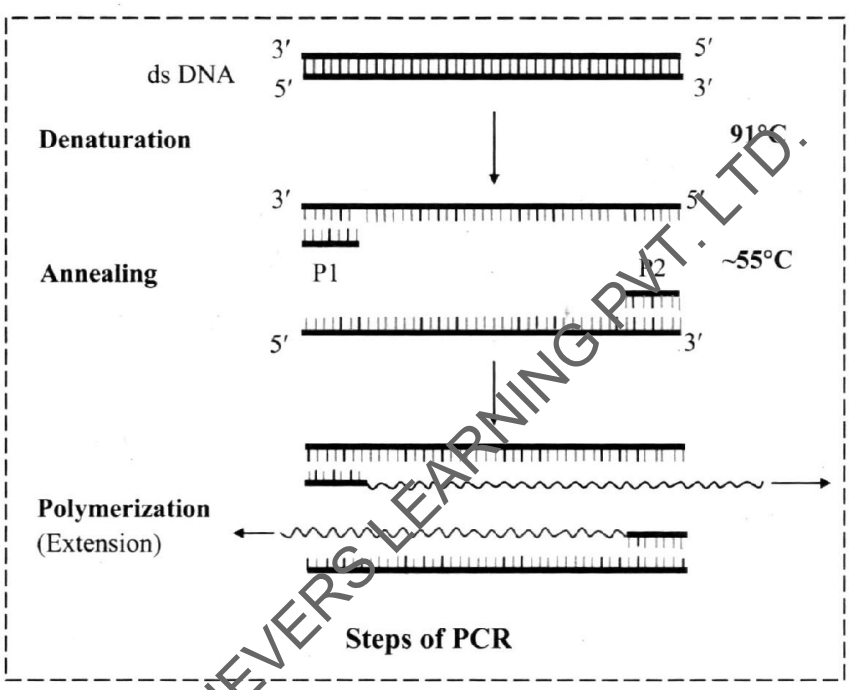
Q.41.Describe the steps that constitute one cycle of PCR.

Ans: One cycle of Polymerase Chain Reaction involves three basic steps:

i) Denaturation

ii) Annealing

iii) Polymerization



Steps of PCR

i) Denaturation :

The desired DNA is heated to a high temperature of about 91°C and forms a single stranded DNA. It results in the separation of the two strands of DNA, each of which would function as a template for the synthesis of a new molecule of DNA. DNA with G-C pair requires a higher temperature.

ii) Annealing :

a. It is the process in which the two primers (oligonucleotides) hybridise to, each of the strands of DNA.

b. This step is carried out at a lower temperature, which depends on the length and the sequence of nucleotides of the primers; the binding usually occurs at the 3' end of the strands.

c. It requires a temperature of 55°C.

iii) Polymerization :

In this step, the Taq polymerase carries out the synthesis of DNA region between the two primers by using deoxyribonucleotides (dNTPs) and Mg^{2+}. The optimum temperature for this polymerization reaction is 72°C. The cycles are repeated number of times according to the need. The products of one cycle functions as the template for the subsequent cycle. Consequently, after 'n' number of cycles, it is possible to generate 2n molecules from a single template molecule.

Q.42.List down the steps involved in PCR.

Ans: The three steps involved in PCR are:

i) Denaturation
ii) Annealing
iii) Polymerization

Q.43.Give the applications of PCR technique. [Oct 2014]

Ans: PCR is used in:

i) DNA cloning
ii) Gene amplification
iii) DNA-based phylogeny or functional analysis of gene.
iv) Diagnosis of hereditary disease.
v) DNA fingerprinting.
vi) Diagnosis of infectious diseases and cancer.

3.6 : Application of Biotechnology in Agriculture – Bt crops

Q.44. What does the letter Bt stand for in Bt toxin ?

Ans: The letter 'Bt' in Bt toxin stands for *Bacillus thuringiensis.*

Q.45.What are cry proteins? Name an organism that produces it. How has man exploited this protein to his benefit ?

Ans: Some strains of bacterium Bacillus thuringiensis produce crystal (cry) protein that have insecticidal property.

i) The bacterium forms the protein crystals at a particular phase of its growth. The protein is not toxic to the bacterium, as it exists in an inactive form, called protoxin.

ii) When it is ingested by an insect, it is converted into an active form by the alkaline pH of the gut; the crystals also get solubilised. The activated toxin binds to the surface of the epithelial cells lining the midgut and create pores through which water enters the cells. It causes swelling and lysis of these cells and eventually leads to death of the insect.

iii) The genes coding for the cry proteins have been isolated from the bacterium and incorporated into several crop plants such as cotton, tobacco, etc. The toxin produced by the transgenic plants makes them resistant to the insect pests.

Q.46. What are Bt crops? Explain in brief about how they are produced with a suitable example.

Ans:i) Bt crops are transgenic insect resistant crops produced through rDNA technology.

ii) *Bacillus thuringiensis* (Bt) is a soil bacterium that produces a protein with insecticidal property.

iii) Bt toxin proteins occur as inactive protoxin.

iv) The 'cry' gene codes for this inactive protoxin.

v) Crops have now been genetically engineered using rDNA technology, by inserting the 'cry' gene in plants.

vi) This 'cry' gene produces inactive protoxin.

vii) After the insects ingest the transgenic crops, they are killed because the inactive protoxin is converted into the active form due to alkaline pH of the insect gut.

viii) Bt toxin gene has been cloned and introduced in many plants to provide resistance to insects without the need of insecticides.

ix) Some ofthe examples ofBt crops are Bt cotton, Bt com, rice, potato, tomato and soyabean.

Q.47.Explain the use of Agrobacterium tumefaciens as a vector in the production of transgenic plants.

Ans:i) ***Agrobacterium tumefaciens*** is a soil bacterium which causes crown gall tumours in dicotyledonous plants.

ii) A gall producing gene (T DNA) occurs in a large plasmid called tumour inducing plasmid or Ti plasmid present in the bacterium.

iii) Due to the ability of the bacterium to insert Ti plasmids into the nuclear genome of the infected plant, gene transfer in higher plants through Ti-plasmids can be achieved.

iv) Tumour producing gene becomes the marker gene.

v) Cry gene from B. thuringiensls or Nif gene (N_2 fixing gene) from *Rhizobium* is cloned inside *A. tumefaciens* and then transferred into other plant.

vi) Many genetically modified plants (eg. Flavr Savr tomato, golden rice) are produced using *A. tumefaciens.*

Q.48.What is golden rice ?

Ans: Golden rice is genetically engineered rice with greater pro-vitamin A (β -carotene) content.

Q.49. Give some examples of genetically modified plants.

Ans:i) **Bt Cotton :** Resistant to insect pests.

ii) **Flavr savr variety of tomato :** Improved shelf life and taste.

iii) **Golden rice:** Enriched with vitamin A.

iv) **Transgenic tobacco:** Resistant to herbicides.

v) **Transgenic potato:** high protein levels.

Q.50. Write a short note on Flavr Savr variety of tomato.

Ans:i) In Flavr Savr variety of tomato, the expression of a native gene that codes for the enzyme. polygalactouranase, has been blocked. The enzyme is responsible for degradation of pectin during fruit ripening. In the absence of this enzyme, the fruit ripening or softening is delayed and hence, the fruit remains fresh for longer periods. .

ii) This transgenic tomato variety has the following desirable qualities:

a) The fruits of this variety remain fresh and retain their flavour much longer than the other normal varieties.

b) They have an increased quantity of total soluble solids.

c) The fruits have a superior taste.

Q.51. Which is the most widely used vector for producing Bt crops ?

Ans: Soil bacterium *Agrobacterium tumefaciens* which causes gall tumours in dicotyledons plants is the most widely used vector for producing Bt crops.

Q.52. What are the different ways of increasing food production?

Ans: The different ways of increasing food production are :

i) Use of chemical fertilizers and pesticides.

ii) Use ofbio-pesticides and bio-fertilizers (organic farming)

iii) Use of genetically engineered crop plants, i.e. transgenic plants or Bt crops.

3.7 : Biosafety Issues

Q.53. Define the following terms :

i) Biopatent

ii) Biopiracy

Ans:i) Biopatent

A biopatent is a patent granted by the government to the inventor for biological entities and for products obtained from them.

ii) Biopiracy

Biopiracy is the unlawful exploitation of bio-resource, already awarded 'biopatent and also biopatenting of bio-resource of other nation without proper permission of the concerned nation.

Q.54. Write a short note on biopatent. **[Mar 2010]**

Ans:i) A biopatent is a patent granted by the government to the inventor for biological entities and for products obtained from them.

ii) The biopatents are awarded for GM strains of micro-organisms, plants and animals, cell lines, DNA sequences or proteins encoded by them, different industrial processes, various biotechnological procedures and products.

iii) Biopatents provide incentive for new inventions and discoveries. They protect intellectual property of an inventor. They also encourage technological innovations, investments and development of beneficial products.

iv) However, some biopatents have been opposed like commercialization of hybrid crops resutling from patent protection of seeds. Many companies invested in hybrid plants and gained control of world markets. This adversely affected farmers world wide.

v) Two important aspects ofbiopatents are as follows :

a. Biopatents should be granted carefully, 'fairly and impartially, because there is unlimited scope, money and industrial power involved in the future of biotechnology.

b. Legal, ethical, economical and social implications of modern biotechnology should be thoroughly examined before granting a biopatent. This ensures that biotechnology products contribute in a positive way.

Q.55. What is a 'biopatent'? Give any 'two' examples. **[Mar 2014]**

Ans: i) A biopatent is a patent granted by the government to the inventor for biological entities and for products obtained from them.

ii) e.g. Basmati rice and Turmeric.

Q.56 Discuss biosafety issues.

Ans:i) Genetic modification of organisms has unpredictable effects when such organisms are introduced into the ecosystem.

ii) Cross pollination between GM plants and wild varieties leads to contamination of gene pools of wild varieties.

iii) Consumption of GM foods may lead to allergies.

iv) GM microbes may escape from the laboratory and prove to be hazardous.

v) Manipulation of living organisms requires regulation.

vi) Biopiracy and biopatent issues are also included under biosafety issues.

vii) Biopiracy is practised by developed countries by patenting the knowledge and bio-resources of underdeveloped countries and enjoying immense profits.

viii) A biopatent is a patent granted by the government to the inventor for biological entities, processes and products.

ix) Patent gives the owner exclusive rights to use the resource, process or market the product and earn profits.

x) Biopiracy is the biopatenting of the bioresource of the other nation without proper permission of the concerned nation or unlawful exploitation and use ofbioresource without giving compensation.

Q.57. What is biopiracy? Give any two examples. **[Sep 2008]**

Ans: Biopiracy is the unlawful exploitation of bio-resource, already awarded biopatent and also biopatenting of bio-resource of other nation without proper permission of the concerned nation;

Cases of biopiracy and unfair patenting :

i) Texmati case :

A strain of Basmati rice was patented by Texas based company Rice Tee. This patenting was illegal and unethical as Basmati is actually communal property of rice growers in the northern sub- Himalayas in India. India fought a long legal battle after which the patent was cancelled.

ii) Turmeric and Neem :

Since ancient times, Indians have been using Haldi (Turmeric powder) as antiseptic for healing wounds and Neem for killing pests and medicinal purposes. However, American companies have patented Turmeric, Neem and many medicinal plants of India. After a long legal battle, most of the patents have been revoked.

Q.58. What leads to biopiracy ?

Ans:i) For proper and lawful working of biopatent, the nation should be rich in bio-diversity, people residing there should have traditional knowledge and the nation should also have sufficient financial resources.

ii) However, it is generally observed that industrialized nations are rich in financial resources and technology but lack bio-diversity, whereas developing countries are rich in bio-diversity and traditional knowledge but are short of financial resources and advanced technology. These situations lead to biopiracy.

iii) Industrialized nations have always been enjoying immense profits by patenting the indigenous biomedical knowledge and bioresources of third world communities without paying any compensation to the indigenous group who originally developed such knowledge.

Additional Theory Questions

Q.1. Describe the mechanism/procedure/process of r-DNA technology. ***Refer Q.12.***

Q.2. What is bacteriophage? Explain the lytic cycle Of bacteriophage with a suitable diagram. ***Refer Q.22 and 23.*** **[Oct 2013]**

Q.3. What is palindrome in DNA ? [Mar 2013] ***Refer Q.27.***

Q.4. Explain Briefly : PCR ***Refer Q.39, 40, 42.***

Q.5. Describe gene amplification (PCR). ***Refer Q.38.***

Q.6. What are the applications of PCR? ***Refer Q.43***

Q.7. Draw a neat labelled diagram showing steps ofPCR. **[Mar 2013]**

Refer Q.41

Q.8. Explain the insecticidal property of cry proteins. *Refer Q.45*

Q.9. Explain different biosafety issues which may arise due to genetically modified (GM) organisms. *Refer Q.56 (i - v)* **[Oct 2014]**

Q.10.Mention some cases ofbiopiracy and unfair patenting. *Refer Q.57 (i) (ii)*

Quick Review

- **Steps involved in rDNA technology**

Isolation of genomic DNA from donor.
↓
Fragmentation of donor DNA by restriction endonucleases.
↓
Screening for desired gene.
↓
Inserting them into cloning vector.
↓
Formation of rDNA.
↓
Introducing recombinant vector into host cell.
↓
Culturing to obtain multiple copies.
↓
Transformation of host cell.

- **Tools used in rDNA technology**

→ Enzymes
  → Restriction endonucleases
  → DNA ligase
  → Reverse transcriptase
  → DNA polymerase
  → Alkaline phosphatases
→ Vectors
  → Plasmid
  → Bacteriophage
  → Artificial DNA
  → Cosmid
→ Desired gene
e.g. Nif gene, insulin gene, etc
→ Host
e.g. Bacteria, yeast, etc

- **Three essential steps in PCR technique**
  → Heat denaturation (91°C)
  → Annealing (55°C)
  → Polymerization (72°C)

- **Scientists and their contribution**

No.	Scientist	Contribution	Year
i)	Peter Lobban and A. Date Kaiser (at Dept. of Biochemistry, Stanford University)	First proposed recombinant DNA technique.	–
ii)	Stanley Cohen and Herbert Boyer	Successfully linked a gene coding for antibiotic resistance with a native plasmid of *salmonella typhimurium* with vector plasmid and then cloning it in *E.coli.*	1972
iii)	Barbara McClintock	Discovered first transponsons in Maize. Awarded Nobel prize for the same in 1983.	1948
iv)	Joshua Lederberg (American Molecular biologist	Introduced the term ' plasmid'.	1952
v)	Bolivar and Rodriguez	Discovered plasmid pBR 322 (designated it 322)	–
vi)	Steward Linn and Werner Arber	Isolated 2 enzymes which restricted the growth of bacteriophage in bacterium *E. coli*	1963
vii)	Temin and Baltimore	cDNA library. cDNA is a complementary DNA. It is produced using mRNA by a process called reverse transcription. This process is called " Teminism".	
viii)	Kary Mullis	Developed the process of gene amplification called PCR Awarded Nobel prize in chemistry for the same in 1993	1983

Multiple Choice Questions

1. Biotechnology is
- a) application of organisms and/or their enzymes and biological systems to manufacture industrial products useful to mankind.
- b) application of organisms to study, genetics.
- c) a science to culture bacteria only.
- d) a science of enzymes only.

2. Recombinant DNA, technique was first proposed by
- a) Herbert Boyer
- b) Stanley Cohen
- c) Peter Lobban
- d) Bolivar

3. The first step in recombinant DNA technology is
- a) isolation of DNA.
- b) insertion of DNA.
- c) joining of DNA.
- d) duplication of DNA.

4. Plasmid is a
- a) fungus
- b) plastid
- c) part of plasma membrane.
- d) extra chromosomal DNA in bacterial cell.

5. A commonly used plasmid from *E.coli* in genetic engineering is
- a) YEP
- c) pBR 322
- c) M13
- d) pUC 13

6. The useful enzymes in recombinant DNA technology are
- a) endonucleases
- b) ligase
- c) alkaline phosphatase
- d) all of these

7. Which of the following is not used as a vector in genetic engineering ?
- a) Plasmid
- b) Cosmids
- c) Bacteriophages
- d) passenger DNA

8. DNA element with ability to change its position is called
- a) cistron
- b) transposon
- c) intron
- d) recon

9. Enzyme that cuts DNA is
- a) DNA polymerase
- b) DNA ligase
- c) DNA lyase
- d) restriction endonuclease

10. Polymerase chain reaction is most useful in
a) DNA synthesis
b) DNA amplication
c) protein synthesis
d) amino acid synthesis

11. Plasmid are extra-chromosomal genetic material of
a) bacteria b) virus
c) algae d) amoeba

12. Molecular scissor is
a) restriction endonuclease
b) helicase
c) urease
d) peptidase

l3. The biological scissor is **[Mar 2014]**
a) restriction endonuclease
b) gyrase
c) DNA ligase
d) helicase

14. The source of Taq polymerase used in PCR is a
a) thermophilic fungus
b) mesophilic fungus
c) thermophilic bacterium
d) halophilic bacterium

15. Cloning can be done invitro, via
a) polymerase chain reaction
b) gel electrophoresis
c) transposons
d) lambda phage

16. The first transposons were discovered in
a) com b) wheat
c) nee d) yeast

17. Bacteriophage Ml3 is with
a) ssDNA b) dsDNA
c) ssRNA d) dsRNA

18. In EcoRI, Eco stands for
a) Eco friendly b) Economic
c) *E. coli* d) Extra coenzyme

19. In nomenclature of RENs (restriction endonucleases) Hind III, III stands for **[Mar 2013]**
a) genus name
b) species name,
c) order of discovery
d) strain of the organism

20. The molecular knives of DNA are
a) ligases b) polymerases
c) endonucleases d) transcriptases

21. Transgenic plants are produced by
a) inducing gene mutation.
b) arresting spindle fibre formation.
c) deleting sex chromosomes.
d) introduction of foreign genes.

22. Bacterium commonly used in plant genetic engineering is
a) Agrobacterium
b) Cyanobacteria
c) Bacillus subtilis
d) Salmonella typhi

23. Restriction endonucleases cleave the DNA molecule by hydrolyzing
a) H-bonds
b) phosphodiester bonds
c) OH-bonds
d) peptide bonds

24. PCR technique was developed by
a) Stanley Cohen
b) Herbert Boyer
c) W. Arber
d) K. Mullis

25. A thermostable DNA polymerase can withstand temperature up to
a) 100°C b) 114°C
c) 214°C d) 94°C

26. Bacillus thuringiensis (Bt) strains have been used for designing novel
a) Bio-metallurgical technique
b) Bio-mineralization processes
c) Bio-insecticidal plants
d) Bio-fertilizers

27. Which of the following improved trait present in Golden rice has made it a transgenic crop of the future ?
a) high lysine (essential amino acid) content
b) insect resistant
c) high protein content
d) high vitamin A content

28. Transgenic crop contains
a) gene for resistance to antibiotics.
b) protein produced by the gene.
c) enzymes produced by the gene for antibiotics.
d) all of these

29. The gene which was used to produce insect resistant cotton plant, was taken from
a) Anabaena azollae
b) Agrobacterium tumefaciens
c) Bacillus anthracis
d) Bacillus thuringiensis

30. Flavr savr is genetically modified
a) cotton b) rice
c) tomato d) potato

31. The enzyme affecting the shelf life of flavr savr tomato is **[Mar 2013]**
a) galactosidase
b) trans acetylase
c) permease
d) polygalactouranase

32. Golden rice will help in
a) producing petrol-like fuel.
b) pest resistance.
c) herbicide tolerance.
d) alleviation of vitamin A deficiency.

33. Bt gene which produces protein toxic to insect larvae is
a) Fry b) cry
c) Trp d) Crp

34. Jumping genes are also known as
a) transversion b) transduction
c) transposons d) transformation

35. A GM crop is
a) irradiated crop.
b) transgenic crop.
c) raised on green manure.
d) a green manure.

36. cDNA library can be prepared by
a) RNA polymerase
b) DNA polymerase
c) Restriction enzyme
d) Reverse transcriptase

37. c-DNA means **[Oct 2014]**
a) coiled DNA
b) cytoplasmic DNA
c) complementary DNA
d) circular DNA

38. Which of the following bacteria IS a good vector in genetic engineering ?
a) Agrobacterium tumefaciens
b) Bacillus thuringiensis
c) Salmonella typhimurium
d) Bacillus amyloliquefaciens

39. Introduction of a transgene
a) can produce a protein product.
b) can alter an existing biosynthetic pathway.
c) both a) and b)
d) none of these.

40. Restriction endonucleases are most widely used in r-DNA technology because they are obtained from
a) plasmids
b) all prokaryotic cells
c) bacteriophage
d) bacterial cells

41. Fragments of DNA formed after treatment with endonucleases are separated by the technique of
a) polymerase chain reaction
b) southem blotting
c) colony hybridisation
d) electrophoresis

42. Biopatents are granted to
a) DNA sequences
b) microbial strains
c) proteins
d) all of these

43. Which of the following risks are associated with genetically modified food ?
a) Toxicity
b) Allergic reaction
c) Antibiotic resistance in microorganisms present in alimentary canal.
d) All of these

44. Ti plasmids are present in
a) *Bacillus thuringiensis*
b) *Agrobacterium tumefaciens*
c) *Haemophilus injluenzae*
d) *Escherichia coli*

45. Crystals of Bt toxin produced by some bacteria do not kill the bacteria themselves because
a) bacteria are resistant to strain.
b) toxin is immature.
c) toxin is inactive.
d) bacteria encloses toxin in a special sac.

46. The most common form of transposons humans
a) Alu b) pBR 322
c) Cry d) Trp

Answer Keys

1. a)	**2.** c)	**3.** a)	**4.** d)	**5.** b)	**6.** d)	**7.** d)	**8.** b)	**9.** d)	**10.** b)
11. a)	**12.** a)	**13.** a)	**14.** c)	**15.** a)	**16.** a)	**17.** a)	**18.** c)	**19.** c)	**20.** c)
21. d)	**22.** a)	**23.** b)	**24.** d)	**25.** d)	**26.** c)	**27.** d)	**28.** d)	**29.** d)	**30.** c)
31. d)	**32.** d)	**33.** b)	**34.** c)	**35.** b)	**36.** d)	**37.** c)	**38.** a)	**39.** c)	**40.** d)
41. d)	**42.** d)	**43.** d)	**44.** b)	**45.** c)	**46.** a)				