DAY THIRTY FOUR

Biotechnology : Principles and Its Applications

Learning & Revision for the Day

- Principles of Biotechnology
- Recombinant DNA Technology
- Process of Recombinant DNA Technology
- Downstream Processing
- Applications of Biotechnology in Agriculture
- Applications of Biotechnology in Healthcare
- Transgenic Animals
- Biosafety Issues

Biotechnology is the use of organisms, their parts or processes for the manufacture or production of useful or commercial substances and for the provision of services such as waste treatment.

According to European Federation of Biotechnology (EFB), the integration of natural science and organisms, cells, parts thereof and molecular analogues for products and services constitutes biotechnology.

Principles of Biotechnology

- The two core techniques that enabled the birth of modern biotechnology are
  - **Genetic engineering** is the technique of altering the nature of genetic material and/or introduction of it into the host organisms to change its phenotype.
  - **Sterilisation techniques** to facilitate the growth and multiplication of only the desired microbes or cells in large number under sterile conditions for the manufacture of biotechnological products.
- The techniques of genetic engineering include

(i) Creation of recombinant DNA (*r*DNA) (ii) Use of gene cloning

(iii) Gene transfer

Recombinant DNA Technology

- It is also known as genetic engineering. It is the most useful technique for creating deliberate modification in an organisms genome or particular gene.

- Through this technology, gene transfer between distantly related organisms such as humans, bacteria or plants has been made possible.
- If these genes integrate permanently into the desired organism, they can be transferred to offspring and the resulting organism is said to be transgenic or recombinant organism. The main tools of recombinant technology are

1. Enzymes

Restriction endonucleases, polymerases and ligases are the main enzymes used in genetic engineering.

(i) **Restriction endonucleases** cut a DNA molecule within certain specific sites that have particular base sequence. Therefore, these are also known as **molecular scissors,** e.g. Restriction enzyme *Hae* III cuts DNA, wherever it recognises the sequence $\frac{5'\ GGCC\ 3'}{3'\ CCGG\ 5'}$. Cut is made between adjacent G and C.

- The restriction enzymes *Eco* RI, *Bam* II and *Hin*d III are used in recombinant DNA technology to produce cuts at specific site of desired DNA.
- The first restriction endonuclease isolated was *Hin*d III.
- About 900 restriction enzymes are known that have been isolated from 230 strains of bacteria.
- Typical restriction site is 4-6 nucleotide base pairs (bp) long.
- Restriction enzymes are palindromes, i.e. they read the same forwards as well as backwards (e.g. bob, racecar)

5′ — GTTAAC — 3′
3′ — CAATTG — 5′

Examples of Restriction Enzymes

Name	Source	Site	Type of End
Hpa I	*Haemophilus parainfluenzae*	5′ GTT - AAC 3′ 3′ CAA - TTG 5′	Blunt
Ssp I	*Sphaerotilus* species	5′ AAT - ATT 3′ 3′ TTA - TAA 5′	Blunt
Pst I	*Providencia stuartii*	5′ CTGCA - G 3′ 3′ G - ACGTC 5′	Sticky
*Hin*d II	*Haemophilus influenzae*	5′ GTC - GAC 3′ 3′ CAG - CTG 5′	Blunt
Eco RI	*Escherichia coli*	5′ G - AATTC 3′ 3′ CTTAA - G 5′	Sticky
Hae III	*Haemophilus aegyptius*	5′ GG - CC 3′ 3′ CC - GG 5′	Blunt
Bam I	*Bacillus amyloliquefaciens*	5′ GGAT - CC3′ 3′ CCTA - GG 5′	Sticky

(ii) **Ligases** help in sealing gaps in DNA fragments by forming phosphodiester bonds. These are also known as molecular glue or binder, e.g. T_4 DNA ligase.

(iii) **Polymerases** are used in synthesising copy of DNA on complementary DNA, e.g. DNA polymerase, reverse transcriptase.

(iv) **Alkaline phosphatases** cut-off phosphate group from end of linearised circular DNA to check its recircularisation.

2. Cloning Vectors

- The vectors are DNA molecules that can carry a foreign DNA segment and replicate inside the host cell. These are also known as vehicles for cloning.
- Vectors may be plasmids, bacteriophages, cosmids, phagemids, Yeast Artificial Chromosomes (YACs), Bacterial Artificial Chromosomes (BACs), etc.
- The following features are required to facilitate cloning in a vector
  - Origin of replication (*Ori*)
  - Selectable marker
  - Presence of recognition site
  - Small size of vector.

(i) Plasmid Vectors

- These are extrachromosomal, self-replicating, usually circular, double-stranded DNA molecules, found naturally in many bacteria and also in some yeast.
- The plasmid molecules may be present in 1 or 2 copies or in multiple copies (500-700) inside the host organism.
- The naturally occurring plasmids have been modified to serve as vectors in the laboratory, e.g. pBR322 is an ideal plasmid vector which can be easily manipulated.
- It was the first artificial cloning vector constructed in 1977 by **Boliver** and **Rodriguez**.
- In the name P signifies plasmid, B and R are the two initials of the scientists who developed it.
- It contains **origin of replication** (*ori*) which allows production of multiple copies per cell.
- It has the two selectable markers (antibiotic resistance genes), i.e. tetracycline, tet^R and ampicillin, amp^R.
- It also possess unique recognition sites or cloning sites for 12 restriction enzymes (endonucleases). Two unique sites, *Pst* I and *Pvu* I are located within the amp^R gene and *Bam* HI, *Sal* I within tet^R gene, etc.

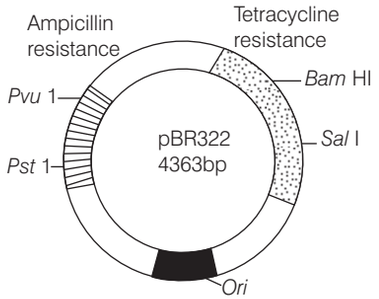


Diagram showing essential features of plasmid pBR322

(ii) Bacteriophage DNA

- Bacteriophage DNA is used for preparing a genomic library of an eukaryote having quite large DNA fragments or even whole genomes.
- The requirements of cloning are fulfilled by lambda (λ) phage derivatives used for transferring the genetic material from one bacterium to other. In these vectors cloning of 20-25 kb is possible.
- These consist of linear double-stranded DNA molecules, which have been engineered in the way that their lytic cycle is possible but lysogenic cycle is not possible.
- The **lambda phage genome** is of about 50 kbp circular DNA.
- It follows either a lytic path or a lysogenic path. Lytic path may be switched towards the lysogeny and *vice-versa*.
- The lambda cloning vectors are of two types
  - The **insertion vector** accepts inserts only 12 kbp long at a single multiple cloning site λ *gt* 10 and λ *gt* 11 vectors.
  - The **replacement vector** which accepts inserts 9-23 kbp long with the involvement of replacement of a non-essential part (stuffer) of genome, e.g. EMBL3 and EMBL4 vectors.

(iii) Cosmids

These are the vectors, which can accommodate DNA segments upto 45 kbp. These are actually plasmid particles and 'cos' sites of lambda phage.
The cosmids allow the packaging of DNA in phage *in vitro*, thus, permitting their purification. Like plasmids, these cosmids also can perpetuate in bacteria.

(iv) Phagemids

These are the plasmids with a fragment of filamentous phage DNA. The phagemids can generate multiple copies of one strand and the associated DNA inserted in it.

(v) Ti-Plasmid

It is a circular plasmid that often, but not always, is a part of the genetic material to plants. It has 196 genes that code for 195 proteins.

- It is 206-479 nucleotides bp in length, the GC content is 56% and 81% of the material is coding genes. There are no pseudogenes.
- The modification of Ti-plasmid is very important in the creation of transgenic plants.

(vi) Bacterial Artificial Chromosome (BAC) Vectors

- These are vectors based on natural, extrachromosomal plasmid of *E. coli*, the fertility or F-plasmid.
- A BAC vector contains genes for replication and maintenance of the F-factor, a selectable marker and cloning sites.
- These vectors can accommodate upto 300-500 kb of foreign DNA and are also being used in genome sequencing projects.

(vii) Yeast Artificial Chromosome (YAC) Vectors

- These are used to clone DNA fragments of more than 1 Mb in size, therefore, they have been exploited extensively in mapping of large genomes, e.g. in the human Genome Project (hGP).
- These vectors contain the telomeric sequence, the centromere and the autonomously replicating sequence from yeast chromosomes.
- They also contain restriction enzyme sites and genes which act as selectable markers in yeast.

3. Competent Host

It is essential requirement for transformation with recombinant DNA. Many kinds of host cells, including *E. coli,* yeast, animal and plant cells are available for genetic engineering.

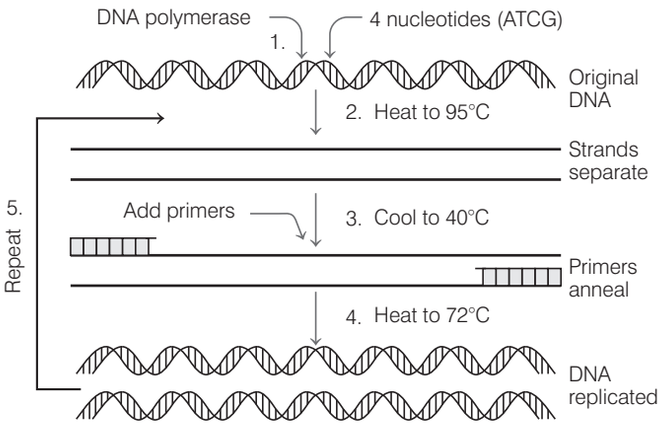
- In animals, the term transformation is replaced by the term **transfection**.
- Other methods to introduce foreign DNA into host cells are briefly described below
  - (a) **Microinjection** involves direct injection of recombinant DNA into the nucleus of animal cell using microneedles or micropipettes.
  - (b) **In electroporation** electrical impulses induce transient pores in the plasmalemma through which DNA molecules enter the cells.
  - (c) **Direct DNA injection** into the skeletal muscle led to the possibility of using gene as vaccines.
  - (d) **In gene gun or biolistics** DNA coated onto microscopic pellets is literally shot into target cells. It is mainly developed for plants.

Process of Recombinant DNA Technology

Recombinant DNA technology involves the following steps

1. **Isolation of DNA** in pure form is necessary for the reaction of restriction enzymes. Enzymes like lysozyme, cellulase, chitinase are involved in this step.
2. **Fragmentation and separation of DNA** is carried out by incubating the purified DNA molecules with suitable restriction enzymes to produce DNA fragments. These fragments can be separated by a technique known as **gel electrophoresis**.
3. **Amplification** of the DNA/gene of interest refers to the process of making multiple copies of the DNA segment *in vitro,* using **Polymerase Chain Reaction** (PCR).

- The PCR process was designed by **K Mullis** in 1983 for which he won Nobel Prize in 1993.
- **PCR** involves three mains steps
  (a) Denaturation (b) Primer annealing
  (c) Extension of primers.
- The double-stranded DNA is denatured by using high temperature. Two sets of chemically synthesised primers are used in this process.



PCR Technique

4. **Ligation** of amplified DNA fragment with the vector forms recombinant DNA (*r*DNA). *r*DNA is made in first meiotic prophase by the process of crossing over.
5. **Transfer of recombinant DNA** into host is done by **microinjection method**. **Gene gun** or **Biolistics** are suitable method for cells transformation.
6. **Culturing of transgenic cell** is done on a suitable medium to make clones.
7. **Extraction of desired product** is done by suitable procedure like by using **bioreactors.**

Downstream Processing

- It is a series of processes, which are to be followed before the extracted product is ready for marketing.
- It involves two main processes, i.e. separation and purification.
- The product is then formulated with suitable preservatives and undergoes clinical trials. This process is performed in large vessel called as bioreactors.
- **Bioreactor** is a kind of vessel, in which raw materials are biologically converted into specific products by microbes, plant and animal cells and/or their enzymes.
- The most commonly used bioreactor is of stirring type.

A bioreactor has following components
(a) An agitator system
(b) An oxygen delivery system
(c) A foam control system
(d) A temperature control system
(e) pH control system
(f) Sampling ports.

- **Random Amplification of Polymorphic DNA** (RAPD) is a type of PCR reaction, but the segments of DNA are randomly amplified.
- **Restriction Fragment Length Polymorphism** (RFLP) is a technique in which organisms may be differentiated by the analysis of pattern derived from DNA fragments. RTLBs are used as molecular marker, in DNA fingerprinting technique, etc.

Applications of Biotechnology in Agriculture

- Biotechnology applied in agriculture processes, helps in reduction of duration of breeding programmes, development of new hybridisation methods, formation of transgenic crops.
- Genetically modified plants are useful in making the plants more tolerant to diseases, pests and abiotic stresses like cold, heat, drought, etc.

Production of Pest Resistant Plants

- ***Bt* cotton** is produced by a bacterium called *Bacillus thuringiensis* (*Bt* for short). *Bt* toxin gene has been cloned from the bacteria and is expressed in plants to provide resistance to insect without the need for insecticides, e.g. *Bt* cotton, *Bt* corn, rice, tomato, potato and soybean, etc.
- The gene (*cry* genes) encoding the protein (toxin) are isolated from the bacterium and incorporated into several crop plants. There are a number of *cry* genes, e.g. *cry* I Ac, *cry* II Ab, *cry* I Ab, etc.
- ***Bt* brinjal** is a transgenic brinjal (also known as an egg plant or aubergine), created by inserting a crystal protein gene (*cry* I Ac) from the soil bacterium *Bacillus thuringiensis* into the genome of various brinjal varieties.
- **Protection against nematodes** is done in tobacco plants which are infected by *Meloidogyne incognita*. **RNA interference** (RNA*i*) is a process applied to prevent this infestation. RNA*i* involves silencing of a specific *m*RNA due to a complementary *ds*RNA molecule that binds to and prevents translation of the *m*RNA silencing.

Production of Improved Varieties

- **Golden rice** a variety of *Oryza sativa* rice produced through genetic engineering to biosynthesise β-carotene, a precursor of pro-vitamin-A in the edible parts of rice.
- Golden rice was developed as a fortified food to be used in areas, where there is a shortage of dietary vitamin-A.
- **Golden rice 2** can produce upto 23 times more β-carotene than the original variety of golden rice. Golden rice was created by scientist Ingo Potrykus of the Institute of Plant Sciences at the Swiss Federal Institute of Technology.
- ***Flavr Savr*** an improved tomato variety is developed by the use of antisense RNA technology. The enzyme polygalacturonase, which damages pectin is deactivated resulting in increased shelf life.
- **Canola** is the variety of either rapeseed (*Brassica napus* L.) or field mustard (*Brassica campestris L.* or *Brassica rapa var.*). Its seeds are used to produce edible oil, suitable for consumption by humans and livestock.

Applications of Biotechnology in Healthcare

In healthcare, biotechnology has tremendous applications. Some important ones are given here

- **Genetically engineered insulin** was prepared in 1983 by Eli Lilly, an American Company. They prepare two DNA sequences coding for chain-A and B of human insulin molecule and introduce it into plasmids of *Escherichia coli* to produce insulin.
- **Gene therapy** is a collection of methods that allows correction of a gene defect diagnosed in a child/embryo.
- Correction of genetic defect involves delivery of a normal gene into the individual or embryo to take over the function of and compensate for the non-functional gene.
- The first clinical gene therapy was done in 1990 in a 4 years old girl with Adenosine Deaminase (ADA) deficiency.
- **Molecular diagnostics** help in early diagnosis of disease, e.g. recombinant DNA technology, Polymerase Chain Reaction (PCR) and Enzyme Linked Immuno Sorbent Assay (ELISA).
- **Recombinant vaccines and monoclonal antibody** have been produced using recombinant DNA and hybridoma technology, respectively.

Transgenic Animals

- These are animals carrying a foreign gene that has been deliberately inserted into its genome.
- Transgenic sheep and goats have been produced to express foreign protein in their milk. Transgenic chicken are now available which synthesise human proteins in the 'white' portion of the eggs.
- Transgenic milk is produced by a sheep named 'Tracy'. This milk has high quantity of proteins, which are required by humans.

Biosafety Issues

- It is mandatory to evaluate the morality of all human activities that might help or harm living organisms.
- The Indian Government has, thus setup an organisation such as **Genetic Engineering Approval Committee** (GEAC) which will make decision regarding the validity of GM research and the safety of GM organisms for public services.
- The modification/usage of living organisms for public services (a food and medicine sources) has created problems with patents and problems of biopiracy arised.
- **Biopatent** is the patent granted by certain companies for the products and technologies that make use of the genetic materials, plants and other biological resources. For example, an American company got patent rights on **Basmati rice** through the US Patent and Trademark office. This allowed the company to sell a new variety of Basmati, in the US and abroad.
- **Biopiracy** refers to the use of the bioresources by multinational companies and other organisations without proper authorisation from the countries and people concerned without compensatory payment.

DAY PRACTICE SESSION 1

FOUNDATION QUESTIONS EXERCISE

1 Modern biotechnology has gained massive popularity as it combines two major techniques which are
(a) genetic engineering
(b) recombinant DNA technology
(c) gene cloning
(d) All of the above

2 Introduction of foreign genes for improving genotype is
(a) tissue culture (b) immunisation
(c) biotechnology (d) genetic engineering

3 Manipulation of DNA in genetic engineering became possible due to the discovery of
(a) restriction endonuclease (b) DNA ligase
(c) transcriptase (d) primase

4 The enzymes which have the ability to recognise and cut specific nucleotide sequences are called as
(a) restriction enzymes
(b) restriction endonucleases
(c) molecular scissors
(d) All of the above

5 Which of the following could be recognition site for the restriction enzyme action?
(a) ATGCAT (b) ATCATC
(c) AAAGGA (d) ATCCTA

6 GAATTC is the recognition site for the restriction endonuclease
(a) *Eco* RI (b) *Hin*d II (c) *Hin*d III (d) *Bam* HI

7 A restriction enzyme breaks between the
(a) base pairs of a DNA molecule
(b) base pairs of DNA-RNA hybrid molecule
(c) sugar and phosphate components of a nucleic acid molecule
(d) exons and introns of a DNA molecule

8 During gene cloning, the enzyme used to join the insert DNA with the plasmid vector is
(a) DNA ligase
(b) restriction endonuclease
(c) alkaline phosphatase
(d) exonuclease

9 The enzyme used to make DNA copies of RNA is
(a) RNA polymerase (b) reverse polymerase
(c) DNA lyase (d) reverse transcriptase

10 The DNA molecule to which the gene of interest is integrated for cloning is called
(a) transformer (b) vector
(c) template (d) carrier

11 Plasmids are suitable vectors for gene cloning because
(a) these are small circular DNA molecules, which can integrate with host chromosomal DNA
(b) these are small circular DNA molecules with their own replication site
(c) these can shuttle between prokaryotic and eukaryotic cells
(d) these often carry antibiotic resistance genes

12 The most important feature in a plasmid to be used as a vector is
(a) origin of replication
(b) presence of a selectable marker
(c) presence of sites for restriction endonuclease
(d) its size

13 An antibiotic resistance gene in a vector usually helps in the selection of
(a) competent cells (b) transformed cells
(c) recombinant cells (d) None of these

14 The Ti-plasmid is often used for making transgenic plants. This plasmid is found in
(a) *Azotobacter*
(b) *Rhizobium* of the roots of leguminous plant
(c) *Agrobacterium*
(d) Yeast as 2 µm plasmid

15 Commonly used vectors for human genome sequencing are ➝ CBSE-AIPMT 2014
(a) T-DNA (b) BAC AND YAC
(c) Expression vectors (d) T/A cloning vectors

16 A cosmid is actually …… particles to which cos sites of …… are attached.
(a) plasmid + filamentous phage
(b) Ti-plasmid + phage
(c) plasmid + λ phage
(d) None of the above

17 Match the following columns.

Column I		Column II	
A.	Plasmid	1.	Virus infecting bacteria
B.	Bacteriophages	2.	Plasmids with fragment of phage DNA
C.	Cosmids	3.	Hybrid vector derived from plasmids
D.	Phagemids	4.	Circular extrachromosomal DNA

Codes

	A	B	C	D		A	B	C	D
(a)	1	4	3	2	(b)	4	1	3	2
(c)	1	4	2	3	(d)	4	3	2	1

18 In addition to *Ori*, the vector requires a ……, which helps in identifying non-transformants and selectively permitting the growth of the transformants.

(a) selectable marker (b) cloning sites
(c) recognition site (d) All of these

19 What type of microparticles are used for the biolistic (gene gun) method of gene transfer ?

(a) Silver or tungsten (b) Gold or tungsten
(c) Platinum or gold (d) None of these

20 The DNA fragments separated on an agarose gel can be visualised after staining with ➝ NEET 2017

(a) bromophenol blue (b) acetocarmine
(c) aniline blue (d) ethidium bromide

21 Which one is a true statement regarding DNA polymerase used in PCR? ➝ CBSE-AIPMT 2012

(a) It is used to ligate introduction DNA in recipient cells
(b) It serves as a selectable marker
(c) It is isolated from a virus
(d) It remains active at high temperature

22 The *Taq* polymerase enzyme is obtained from ➝ NEET 2016

(a) *Thiobacillus ferroxidans* (b) *Bacillus subtilis*
(c) *Pseudomonas subtilis* (d) *Thermus aquaticus*

23 PCR and Restriction Fragment Length Polymorphism (RFLP) are the method for ➝ CBSE-AIPMT 2012

(a) study of enzymes (b) genetic transformation
(c) DNA sequencing (d) genetic fingerprinting

24 DNA fragments generated by the restriction endonucleases in a chemical reaction can be separated by ➝ NEET 2013

(a) polymerase chain reaction
(b) electrophoresis
(c) restriction mapping
(d) centrifugation

25 Continuous addition of sugars in 'fed batch' fermentation is done to ➝ CBSE-AIPMT 2011

(a) obtain antibiotics (b) purify enzymes
(c) degrade sewage (d) produce methane

26 DNA or RNA segment, tagged with a radioactive molecule is called ➝ CBSE-AIPMT 2010

(a) vector (b) probe
(c) clone (d) plasmid

27 In which of the following techniques, DNA probes can be hybridised with RNA fragments?

(a) Eastern blotting (b) Western blotting
(c) Southern blotting (d) Northern blotting

28 Among the following enzymes which one is most essentially required for Polymerase Chain Reaction (PCR) is

(a) RNA polymerase (b) ribonuclease
(c) *Taq* polymerase (d) endonuclease

29 The process of separation and purification of expressed protein before marketing is called ➝ NEET 2017

(a) upstream processing
(b) downstream processing
(c) bioprocessing
(d) post-production processing

30 Stirred-tank bioreactors have been designed for ➝ NEET 2016

(a) purification of product
(b) addition of preservatives to the product
(c) availability of oxygen throughout the process
(d) ensuring anaerobic condition in the culture vessel

31 Which of the following is an insecticidal protein produced by *Bacillus thuringiensis*?

(a) Rop (b) Hir
(c) Cry (d) Both (a) and (c)

32 The trigger for activation of toxin of *Bacillus thuringiensis* is

(a) acidic pH of stomach
(b) high temperature
(c) alkaline pH of gut
(d) mechanical action in the insect gut

33 Genetically modified tobacco plant with *Bt* gene is resistant to

(a) bollworms (b) hornworms
(c) hookworms (d) roundworms

34 Which part of the tobacco plant is infected by *Meloidogyne incongnita*?

(a) Leaf (b) Stem (c) Root (d) Flower

35 RNA interference which is employed in making tobacco plant resistant to *Meloidogyne incognita* is essentially involved in preventing the process of

(a) translation of *m*RNA (b) transcription
(c) replication of DNA (d) splicing of *hn*RNA

36 A transgenic food crop which may help in solving the problem of night blindness in developing countries is

(a) Golden rice (b) *Bt* soybean
(c) *Flavr Savr* tomato (d) Starlink maize

37 Which of the following plants have been genetically engineered to provide herbicide tolerance?

(a) Soybean (b) Maize
(c) Apple (d) Both (a) and (b)

38 Human insulin is being commercially produced from a transgenic species of

(a) *Escherichia* (b) *Mycobacterium*
(c) *Rhizobium* (d) *Saccharomyces*

39 The two polypeptides of human insulin are linked together by ➝ NEET 2016

(a) phosphodiester bonds (b) covalent bonds
(c) disulphide bridges (d) hydrogen bonds

40 *Bt* cotton is a transgenic plant having *Bt* gene derived from

(a) *Bacillus thuringiensis*
(b) *Bacillus tuberculosis*
(c) *Agrobacterium tumefaciens*
(d) *Agrobacterium rhizogenes*

41 Which of the following *Bt* crops is being grown in India by the farmers? ➡ NEET 2013

(a) Cotton (b) Brinjal
(c) Soybean (d) Maize

42 First genetically modified plants commercially released in India is

(a) golden rice (b) slow ripening tomato
(c) *Bt* brinjal (d) *Bt* cotton

43 Genetically engineered bacteria like *E. coli* used for production of

(a) human insulin (b) cortisone
(c) epinephrine (d) thyroxine

44 A permanent cure for the treatment of Severe Combined Immuno Deficiency (SCID) will be

(a) gene therapy
(b) bone marrow transplant
(c) enzyme replacement therapy
(d) monoclonal antibody treatment

45 The DNA fragment used to detect the targeted DNA, is called

(a) DNA chip (b) DNA probe
(c) gel electrophoresis (d) PCR

46 A molecular diagnostic technique which can be used to detect the presence of a pathogen in early stage of infection is

(a) angiography
(b) radiography
(c) enzyme replacement technique
(d) polymerase chain reaction

47 Hybridoma technology for the production of monoclonal antibodies was developed by

(a) Waksman and Woodruff
(b) Cesar Milstein and George Kohler
(c) Adward Jenner and Louis Pasteur
(d) Paven and Perroz

48 Monoclonal antibodies are used in

(a) pregnancy testing
(b) diagnosis of disease
(c) preventing rejection of transplants
(d) All of the above

49 Use of bioresources by multinational companies and organisations without authorisation from the concerned country and its people is called

(a) biodegradation
(b) biopiracy
(c) bioinfringement
(d) bioexploitation

50 A regulatory body working under MoEF for the release of transgenic crop is

(a) NBPGR (b) GEAC
(c) NSC (d) NIPGR

51 In India, the organisation responsible for assessing the safety of introducing genetically modified organisms for public use is

(a) Research Committee on Genetic Manipulation (RCGM)
(b) Council for Scientific and Industrial Research (CSIR)
(c) Indian Council of Medical Research (ICMR)
(d) Genetic Engineering Appraisal Committee (GEAC)

52 Transgenic animal has

(a) foreign DNA in all its cells
(b) foreign RNA in all its cells
(c) foreign DNA in some of the cells
(d) Both (b) and (c)

53 A transgene expression can achieve which of the following?

(a) Prevent expression of a native gene
(b) Modify an existing biosynthetic pathway
(c) Produce a protein that itself produces the phenotype of interest or is the product of interest
(d) All of the above

54 Biopiracy is related to which of the following?

(a) Traditional knowledge
(b) Biomolecules and regarding bioresources
(c) Genes isolated from bioresources
(d) All of the above

55 Identify the incorrect statement.

(a) Bioethics is the unauthorised use of bioresources and traditional knowledge for commercial benefits
(b) Biopatent is the exploitation of bioresources for war purposes
(c) Both (a) and (b)
(d) Rosie, a transgenic cow produced milk enriched with human α-lactalbumin

DAY PRACTICE SESSION 2

PROGRESSIVE QUESTIONS EXERCISE

1 Which of the following is commonly used as a vector introducing a DNA fragment in human lymphocytes?
(a) λ phage
(b) Ti-plasmid
(c) Retrovirus
(d) pBR322

2 *E. coli* cloning vector pBR322 contains restriction sites in the region of amp^R, tet^R genes that codes for the
(a) antibiotic resistance genes
(b) foreign DNA
(c) selection of recombinants from non-recombinants
(d) proteins involved in the replication of the plasmid

3 Which of the following is incorrect for recognition sequences?
(a) Modification by methylation of bases within them prevents restriction of bacterial DNA
(b) They are usually symmetrical sequences of four to eight nucleotides
(c) They signal the attachment of RNA polymerase
(d) Each recognition sequence is cut by a specific restriction enzyme

4 You are attempting to introduce a gene that imparts larval moth resistance to bean plants. Which of the following vectors are you most likely to use?
(a) Phage DNA
(b) Bacterial plasmid
(c) Ti-plasmid
(d) Yeast plasmid

5 What is the criteria for DNA fragments movement on agarose gel during gel electrophoresis?
(a) The larger the fragments size, the farther it moves
(b) The smaller the fragment size, the farther is moves
(c) Positively charged fragments move to farther end
(d) Negatively charged fragments do not move

6 Which of the following steps are catalysed by *Taq* polymerase in a PCR reaction?
(a) Denaturation of template DNA
(b) Annealing of primers to template DNA
(c) Extension of primer end on the template DNA
(d) All of the above

7 Significance of heat shock method in bacterial transformation is to facilitate
(a) binding of DNA to the cell wall
(b) uptake of DNA through membrane transport proteins
(c) uptake of DNA through transient pores in the bacterial cell wall
(d) expression of antibiotic resistance gene

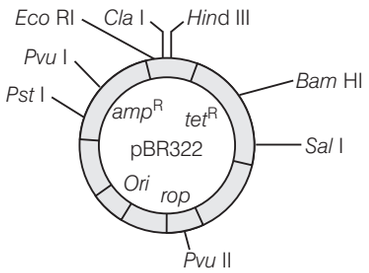
8 What is the advantage of clinical use of humulin over use of conventional ox or pig insulin?
(a) It does not cause immunological problems
(b) It is cheaper for the patient
(c) It is produced by *E. coli* in our intestine
(d) There is no advantage

9 *cry* IIAb and *cry* IAb produce toxins when introduced into plants help in control against
(a) cotton bollworms and corn borer, respectively
(b) corn borer and cotton bollworms, respectively
(c) tobacco budworms and nematodes, respectively
(d) nematodes and tobacco budworms, respectively

10 The first restriction endonuclease type II …*A*…, was isolated by Smith, Wilcox and Kelley from …*B*. It recognised and cut DNA molecules at a particular point, i.e. specific sequence of six base pairs, known as the …*C*… . Here *A*, *B* and *C* can be

	A	*B*	*C*
(a)	*Eco* RI	*Escherichia* RY13	Restriction sequence
(b)	*Eco* RII	*E. coli* R 245	Recognition sequence
(c)	*Hind II*	*Haemophilus influenzae*	Recognition sequence
(d)	*Bam* HI	*Bacilius amyloliquefaciens*	Restriction sequence

11 The figure below is the diagrammatic representation of the *E. coli* vector pBR322. Which one of the given options correctly identifies its certain components(s)?



(a) *Ori* – original restriction enzyme
(b) *rop* – reduced osmotic pressure
(c) *Hind* III, *Eco* RI–selectable markers
(d) amp^R, tet^R – antibiotic resistance genes

12 The colonies of recombinant bacteria appear white in contrast to blue colonies of non-recombinant bacteria because of

(a) insertional inactivation of α-galactosidase in non-recombinant bacteria
(b) insertional inactivation of α-galactosidase in recombinant bacteria
(c) inactivation of glycosidase enzyme in recombinant bacteria
(d) non-recombinant bacteria containing β-galactosidase

13 *Bt* toxin protein crystals present in bacterium *Bacillus thuringiensis*, do not kill the bacteria themselves because

(a) bacteria are resistant to the toxin
(b) bacteria enclose toxins in a special sac
(c) toxins occur as inactive protoxins in bacteria
(d) None of the above

14 Match the following columns.

	Column I		Column II
A.	Gene therapy	1.	Effort to fix functional gene
B.	Humulin	2.	A single-stranded DNA or RNA tagged with a radioactive molecule
C.	Probe	3.	Diagnostic test
D.	ELISA	4.	Diabetes

Codes

	A	B	C	D
(a)	1	4	2	3
(b)	4	2	3	1
(c)	2	3	1	4
(d)	3	1	4	3

15 Match the following columns.

	Column I		Column II
A.	Bacterial viruses	1.	Transformation
B.	Process by which bacteria take up pieces of DNA from the environment	2.	Cloning vector
C.	*Hin*d II	3.	*Haemophilus influenzae*
D.	Vehicle that moves DNA from one organism to another	4.	Bacteriophages

Codes

	A	B	C	D
(a)	2	3	4	1
(b)	1	3	4	2
(c)	4	1	3	2
(d)	1	4	3	2

16 Which of the given statement is correct in the context of observing DNA separated by agarose gel electrophoresis?

(a) DNA can be seen in visible light
(b) DNA can be seen without staining in visible light
(c) Ethidium bromide stained DNA can be seen in visible light
(d) Ethidium bromide stained DNA can be seen under exposure to UV light

17 In electroporation, the cells exposed to high voltage pulse, to temporarily disrupt their membrane. This is done to achieve

(a) increasing uptake of plasmids by bacteria or animal cells
(b) DNA replication
(c) RNA replication
(d) to carry DNA into plant cells

18 Match the following columns.

	Column I		Column II
A.	RNA*i*	1.	Cotton bollworms
B.	ELISA	2.	Early detection of HIV
C.	PCR	3.	*Meloidogyne incognita*
D.	*cry* IAb	4.	Antigen-antibody
		5.	Corn borer

Codes

	A	B	C	D
(a)	3	4	2	5
(b)	4	3	1	5
(c)	2	3	5	4
(d)	5	1	3	2

Directions (Q. Nos. 19-23) *In each of the following questions a statement of Assertion is given followed by a corresponding statement of Reason just below it. Of the statements, mark the correct answer as*

(a) If both Assertion and Reason are true and Reason is the correct explanation of Assertion
(b) If both Assertion and Reason are true, but Reason is not the correct explanation of Assertion
(c) If Assertion is true, but Reason is false
(d) If both Assertion and Reason are false

19 **Assertion** In recombinant DNA technology, human genes are often transferred into bacteria (prokaryotes) or yeast (eukaryotes).
Reason Both bacteria and yeast multiply very fast to form huge population, which express the desired gene.

20 **Assertion** Transgenic plant production is an application of palnt tissue culture.
Reason An organism that contains and expresses a transgene is called transgenic organism.

21 **Assertion** A genetic probe is helpful in the detection of specific DNA sequence.
Reason Genetic probe is radiolabelled *c*DNA which has complementary base sequence, to DNA fragment which is to be detected.

22 **Assertion** *Flavr Savr*, a transgenic tomato remains fresh and retains its flavour for long time.
Reason Production of polygalacturonase enzymes, which degrades pectin was blocked in *Flavr Savr*.

23 **Assertion** Genetic engineering overcomes the drawbacks of traditional hybridisation.
Reason Genetic engineering involves creation of recombinant DNA and introduces the desirable genes into the target organisms.

ANSWERS

SESSION 1

1 (d)	**2** (d)	**3** (a)	**4** (d)	**5** (a)	**6** (a)	**7** (c)	**8** (a)	**9** (d)	**10** (b)
11 (b)	**12** (a)	**13** (b)	**14** (c)	**15** (b)	**16** (c)	**17** (b)	**18** (a)	**19** (b)	**20** (d)
21 (d)	**22** (d)	**23** (d)	**24** (b)	**25** (b)	**26** (b)	**27** (d)	**28** (c)	**29** (b)	**30** (c)
31 (c)	**32** (c)	**33** (a)	**34** (c)	**35** (a)	**36** (a)	**37** (d)	**38** (a)	**39** (c)	**40** (a)
41 (a)	**42** (a)	**43** (a)	**44** (a)	**45** (b)	**46** (d)	**47** (b)	**48** (d)	**49** (b)	**50** (b)
51 (d)	**52** (a)	**53** (d)	**54** (d)	**55** (c)					

SESSION 2

1 (c)	**2** (c)	**3** (c)	**4** (c)	**5** (b)	**6** (c)	**7** (c)	**8** (a)	**9** (a)	**10** (c)
11 (d)	**12** (d)	**13** (c)	**14** (a)	**15** (c)	**16** (d)	**17** (a)	**18** (a)	**19** (a)	**20** (b)
21 (a)	**22** (a)	**23** (a)							