BIOTECHNOLOGY

Time allowed : 3 hours ***Maximum Marks : 70***

General Instructions :

(i) All questions are compulsory.

(ii) There is no overall choice. However, an internal choice has been provided in one question of three marks and three question of five marks. You have attempt only one of the choice in such questions. Questions paper contains four sections - A, B, C and D.

*(iii) Question numbers **1** to **5** are very short answer questions, carrying **1** mark each.*

*(iv) Question numbers **6** to **15** are short answer questions, carrying **2** marks each.*

*(v) Question numbers **16** to **25** are also short answer questions, carrying **3** marks each.*

*(vi) Question numbers **26** to **28** are long answer questions, carrying **5** marks each.*

(vii) Use of calculators is not permitted. However, you may use log tables, if necessary.

QUESTION PAPER CODE 99/1

SECTION A

1. Protein chemists prefer to monitor absorbance of protein fractions at 280 nm as compared to any colourimetric method. Why ? 1

2. Why are eukaryotic hosts preferred for expressing eukaryotic recombinant proteins ? 1

3. What is the mode of action of tissue plasminogen activator (t-PA) ? 1

4. Which of the two methods will be a better indicator of microbial growth — viable plate count or a slide counting chamber, and why ? 1

5. In the presence of glycerol and a high concentration of serum, it is possible to store animal cells for long periods at very low temperatures. Why ? 1

SECTION B

6. Write two distinguishing features of BAC and YAC vectors. 2

7. What is the IUPAC code for G or A ? Write the complementary sequence of the following sequence : 2

5' — ATGAYCGBT — 3'

8. Why is aeration important for microbial growth ? How can proper aeration be achieved in microbial cultures grown in the laboratory ? 2

9. How can single plant cells be isolated and cuttured ? Give two applications of single cell suspension cultures. 2

10. If you wish to scale up cells derived from kidney tissue, what kind of animal culture set-up will you use, and why ? 2

11. You are interested in studying the effect of specific point mutations on the stability of a protein. How will you go ahead to introduce specific point mutations ? 2

12. What is nick translation ? With what aim does a biotechnologist nick translate DNA ? 2

13. If a bacterial culture contains 10^4 cells/ml at t_0 and 10^9 cells/ml after 4 hours, calculate its specific growth rate and doubling time. 2

14. Why are Ti-plasmid based vectors disarmed ? Where is the gene of interest incorporated in this plasmid ? 2

15. What is a continuous culture ? Give one application of setting up continuous cultures in microbial technology. 2

SECTION C

16. Detergent manufacturers supplement their products with subtilisin (a protease). The enzyme is inactivated by bleach. Why is the enzyme rendered inactive by bleach and how can this problem be overcome ? 3

17. What are single nucleotide polymorphisms ? With the help of any two examples explain the relevance of studying SNPs. 3

18. Schematically depict the steps in downstream processing of a microbially produced recombinant insulin. Name an organism used for the commercial production of penicillin. 3

19. How can you obtain virus-free sugarcane plants from virus-infected plants ? Are these plants virus-resistant ? Why or why not ? 3

20. Explain the use of the following in an animal culture laboratory : 3

(i) Laminar air-flow (LAF) hood

(ii) Inverted microscope

(iii) CO_2 incubators

21. Describe the principle of peptide mapping. Who developed this technique ? 3

OR

The relationship between the number of genes and the number of proteins is non-linear. Explain the above statement.

22. Explain how DNA microarray technique can be used to study cellular response to environmen 23. What are edible vaccines ? Give three advantages of developing edible vaccines. Which plant parts will be best suited for expressing the antigenic transgene ? 3

24. Complete the protein purification table given below : 3

Purification procedure	Total protein (mg)	Total activity (units)	Specific activity ———	Purification level
Crude extract	15,000	3,000,000	—	—
Salt precipitation	4,000	2,400,000	—	—
DEAE Chromatography	1,500	1,050,000	—	—
Size exclusion Chromatography	500	800,000	—	—

25. What is the role of erythropoietin ? Why is recombinant human erythropoietin (rHuEPO) preferred over blood transfusion ? Give two reasons. 3

SECTION D

26. What are type II restriction endonucleases (RE) ? Give an example of a type II RE that generates flush ends and the sequence recognised by it. Explain how REs are named. Mention two other enzymes and their utility in cloning experiments. 5

OR

What is a DNA probe ? Explain the principle of Sanger's method of DNA sequencing. Draw the structure of ddCTP.

27. Explain how the "charge relay system" operates in chymotrypsin. Name two other enzymes that use a similar theme. Why do organophosphates inhibit these enzymes ? 5

28. What are the basic steps of a polymerase chain reaction (PCR) ? How can we selectively amplify a DNA fragment ? Write two applications of PCR. 5

OR

How will you select bacterial cells transformed by a recombinant plasmid ? How can E. coli be made competent ? Who developed this method ?

QUESTION PAPER CODE 99

SECTION A

1. Why do hydrophobic regions of a protein form the core of a folded protein ? 1

2. How can self-ligation be avoided in a recombinant DNA construction ? 1

3. Who developed the hybridoma technology ? 1

4. Why is it recommended to work with GRAS organisms ? 1

5. In the presence of DMSO and a high concentration of serum, it is possible to store animal cells for long periods at very low temperatures. Why ? 1

SECTION B

6. Write two distinguishing features of pBR322 and pUC19 vectors. 2

7. What is the IUPAC code for T or C ? Write the complementary sequence of the following sequence : 2

   5' — GACATRTAB — 3'

8. Why is nutrient medium autoclaved before it is used for culturing microbes ? How will you sterilise a heat-labile substance such as an antibiotic solution ? 2

9. What is a callus and how can callus cultures be maintained for prolonged periods? List two applications of callus cultures. 2

10. Patients who are administered OKT3 do not suffer from an acute renal allograft rejection. Why ? 2

11. You are interested in studying the effect of specific point mutations on the stability of an enzyme. How will you go ahead to introduce specific point mutations ? 2

12. Expand 'BLAST'. What kind of analysis can be undertaken with this search tool ? 2

13. If a bacterial culture contains 10^5 cells/ml at t_0 and 10^{10} cells/ml after 4 hours, calculate its specific growth rate and doubling time. 2

14. Why is Bt cotton insect resistant ? Suggest two advantages of growing Bt cotton. 2

15. What is a "fed-batch" culture ? Why is it better than a "batch" culture ? 2

SECTION C

16. Why has sickle-cell trait been selected in populations where malaria is endemic ? What is the molecular basis of sickle cell anemia ? 3

17. What are SNPs ? Indicate the importance of generating SNP maps. 3

18. Schematically depict the steps in down-stream processing of a microbially-produced antibiotic. Name an organism that is used for producing streptomycin. 3

19. How can you obtain virus-free potato plants from virus-infected plants ? Are these plants virus-resistant ? Why or why not ? 3

20. Explain the use of the following instruments in an animal culture laboratory : 3
    (i) CO_2 incubator
    (ii) Centrifuge
    (iii) Inverted microscope

21. You have succeeded in purifying a protein from yeast. Name a technique you would use and the principle behind it for determining the molecular mass of this protein. 3

OR

The relationship between the number of genes and the number of proteins is non-linear. Explain the above statement.

22. Explain how DNA microarray technique can be used to study tissue-specific expression of genes. 3

23. What is meant by molecular breeding ? Explain with examples the type of markers used in screening/selection. 3

24. Complete the protein purification table given below : 3

Purification procedure	Total protein (mg)	Total activity (units)	Specific activity ———	Purification level
Crude extract	20,000	4,000,000	—	—
Salt precipitation	5,000	3,000,000	—	—
DEAE-cellulose Chromatography	1,500	1,000,000	—	—
Size exclusion Chromatography	500	750,000	—	—

25. What is the role of serum in culturing animal cells ? 3

SECTION D

26. Why are restriction endonucleases (RE) called molecular scissors ? Give an example of a type II RE that generates sticky ends and the sequence recognised by it. Why do bacteria produce RE and how do they protect their own DNA from its action? 5

OR

(a) What is a cDNA library ? List two advantages of a cDNA library over a genomic library.

(b) Why are ddNTP used in DNA sequencing ? Draw the structure of ddCTP.

27. The functional properties of a protein are dependent on its 3D structure.

(a) Name the non-covalent interactions involved in making a folded protein.

(b) Differentiate between hydrogen bonds and Van der Waals forces.

(c) What are 'prions' ? Name a disease caused by them. 5

28. What are the basic requirements of a polymerase chain reaction (PCR) ? Why are thermostable polymerases used in PCR ? Give two applications of PCR. 5

OR

(a) What is the principle of blue-white selection for the presence of recombinant plasmids ?

(b) Name two methods of introducing recombinant DNA into host cells.

Marking Scheme — Biotechnology

General Instructions

1 Instruction for drawing up the MS should be followed carefully.

2. If general Instruction have to be given, do so at the beginning of the page intself.

3. Some subject will require specific direction for a particular type of question. Give these at beginning of the concerned question. Don't omit indication of value points, times required for any of the question, even though it may seem obvious.

QUESTION PAPER CODE 99/1

EXPECTED ANSWERS/VALUE POINTS

SECTION "A"

Q.1 Spectrophotometric method is nondestructive (1)

OR

fast (1)

Q.2 Any 2 of the following :

(i) to proper folding

(ii) removal of introns ½+½ (1)

(iii) modification of proteins

Q.3 Dissolves blood clots (1)

Q.4 Viable plate count is better. ½

Slide counting chamber counts both live & dead cells. ½

Q.5 Glycerol prevents formation of ice crystals which damage cells. ½

Serum maintains the integrity of cells ½

SECTION "B"

Q.6 Any two of the following points for each :

	BAC		YAC	
(i)	Host- Bacteria	(i)	Yeast	
(ii)	Clone size - 350 kb	(ii)	1 MB	
(iii)	Ability to replicate in bacteria because of F factor	(iii)	Ability to replicate in yeast because of teleomeric sequence, centromere and ARS.	½×4 = (2)

Q.7 IUPAC code for G or A is 'R' (1)

Complementary sequence - 3 TACTRG/A/CA 5'

(B= G/A/C) (1)

Q.8 For efficient oxygen transfer (1)

Baffle flasks/shakers (1)

Q.9 Isolation from callus Or any other parts ½

by Mechanical or Enzymatic method ½

Any 2 of the following application:

(i) Induction of somatic embryos

(ii) in vitro mutagenesis & mutant selection

(iii) Genetic transformation

(iv) production of secondary metabolites ½+½ = 1

Q.10 Adherent culture using roller bottles/micro carrier beads. (1)

Reasoning: Cells are not mobile and are embedded in connective tissue. (1)

Q.11 Site directed mutagenesis ½

Description /diagrammatically or in points 1½

Q.12 Process involves:

Causing nicks/breaks by DNAse ½

addition of dNTPs to the generated 3' end using DNA polymerase ½

Purpose: To introduce colors/fluorescence by way of nucleotides eg. FISH (1)

Q.13 $\mu = 2.303 \frac{(\log_{10} X - \log_{10} X_0)}{t - t_0} hr^{-1}$ ½

$\mu = \frac{2.303(9-4)}{4} hr^{-1}$

$\mu = 2.8\ hr^{-1}$ ½

Doubling time $= \frac{0.693}{\mu}$ ½

$= \frac{0.693}{2.8} = 0.24$ hr ½

Q.14 Must be disarmed otherwise it will form crown gall (Tumour) Infection (1)

T-DNA (1)

Q.15 Definition : (i) One nutrient is limiting ½

(ii) Before it is exhausted, fresh medium is added, so that it is volumetrically equal to the volume removed for harvesting. ½

Application : Production of biomass and metabolites. (1)

SECTION 'C'

Q.16 Inactivated due to the oxidation of methionine at position 222 (1)

Using site directed mutagenesis (1)

Methionine has been substituted by alanine. (1)

Q.17 Full form: single Nucleotide polymorphism.

Definition: Sequence variation involving a single base. Different individuals have different bases at those positions. (1)

Application (any 2) :

— Medicine

— Forensics/ Criminology

— Population genetics 1+1=2

Q.18 Diagrammatic representation of the downstream processing 2½

Penicillium chrysogenum ½

Q.19 Propagation of plants using meristems collected from virus infected plants (1)

No (1)

They are free from virus particles but are yet virus sensitive. (1)

Q.20 (i) Laminar Air Flow Hood maintains:

asceptic/sterile conditions in a work area to avoid contamination. (1)

(ii) Inverted Microscope: Allows visualization of cells at the bottom of the Petri plate because of an inverted optical system i.e. light source on the top (1)

(iii) Provides conditions closest to the natural environments for animals cells i.e. fixed level of CO_2 high relative humidity, temperature, sterility (1)

Q.21 Diagrammatically/Text.

Steps should include

(i) generation of peptides from protein using trypsin ½

(ii) Paper electrophoresis ½

(iii) Chromatography at 90° to the direction of electrophoresis and separation of peptides based on partition coefficient in the given solvent system (1)

(iv) Visualization of the peptides using ninhydrin. ½

* Ingram ½

OR

Reasons :

(i) Non correlation between mRNA and protein expression (1)

(ii) mRNA can undergo post transcriptional modifications differentially. (1)

(iii) Protein can undergo post translational modification differentially. (1)

Q.22 Indicate through diagram or in points (steps should include).

— Choosing cell population and extracting mRNA.

— Reverse transcribing the mRNA to get cDNA.

— Fluorescent labelling of cDNA

— Hybridization to a DNA microarray.

— Scanning the hybridized array.

— Interpretation of scanned image ½×6 = 3

Q.23 Edible vaccines are those which can be eaten for vaccination/immunization, which are produced using transgenic plants. (1)

* Advantages:

— Easy to store and transport.

— Low cost.

— Easy delivery system. ½×3 = 1½

* Fruits/Tubers (Tomato/sugarbeets, banana) ½

Q.24 * Specific activity units/mg (1)

* crude extract - 200

* salt precipitation - 600

* DEAE chromatography - 700

* Size exclusion chromatography - 1600 ½×4 = 2

Q.25 * Erythropoietin is hormone like substance that stimulates the formation of erythrocytes (1)

Reasons for using rHu EPO

— No donors & transfusion facilities required.

— No risk of disease to the patient. 1 + 1=2

SECTION 'D'

Q.26 R.E. type II recognize a specific DNA sequence and cut within the sequence generating sticky/flush end. (1)

* Alu I - 5 AG CT3
* Sma I - 5 CCC GGG 3 } any 1 ½ +½ = (1)
* Hae III 5 GG CC 3

* Nomenclature with 1 example (1)

* DNA ligase - use ½ + ½
* Alkaline phosphatase - use ½ + ½ (2)

OR

* DNA Probe: Small sequence of DNA that recognizes and binds to its complementary sequence. (1)

* Sanger's Method: Must indicate the following reagents :

— single strand DNA
— A primer with a free 3' - OH.
— DNA polymerase
— dNTPs (1)
— ddNTPs (+ its role) ½

Must include the following steps:

— Primer extension in 4 different tubes each containing a specific ddNTP at low concentration.
— Termination at the point where ddNTP is incorporated.
— Gel electrophoresis
— Autoradiography- + reading of the gel sequence ½×4 = 2

* Structure of any ddNTP - (dideoxy ribose is a must). ½

Q.27 Charge relay system - must include

- Serine 195
- histidine 57 (1)
- Aspartate 102 charge relay should be indicated

* Mechanism of operation.

- Nucleophilic attack of serine O on carbonyl of peptide bond and formation of tetrahedral complex. (1)

(self explanatory diagrams or written points).

* Breakage of peptide bond by water and release of 1 product. (1)

* Acyl-enzyme complex breaks

Any 2 enzymes of the following:

— Trypsin
— Subtilisin ½+½ = 1
— Thrombin
— Acetyl choline esterase

Organophosphates inactivate the serine rendering enzyme inactive. (1)

Q.28 * Basic steps should include
Denaturation ½×3 = 1 ½
Annealing (3)
Extension/ Polymerization ½×3 =1 ½
Explanation/diagram of each step

* Selective Amplification by designing suitable primers to include the sequence which is to be amplified. (1)

* Any 2 of the following application:

— DNA fingerprinting/ Forensic Science
— Detection of infective agents.
— Identification of genetic diseases. ½+½= 1

OR

Description of any 1 of the 2 methods for selection of Recombinants:

— Antibiotic resistance/ (Insertional inactivation)
— Blue white selection (3)

* Method of making competent cells 1½

* Mandel and Higa ½

QUESTION PAPER CODE 99

EXPECTED ANSWERS/VALUE POINTS

SECTION "A"

1. Water forces the hydrophobic regions of a protein out of solution to minimize the surface of contact thus allowing minimal breakage of hydrogen bonds (a favorable interaction). (1)

2. Any one of the two : (1) by treating the digested vector with alkaline phosphatase (1)
(2) Using 2 different restriction enzymes for cloning method.

3. Caesar Milstein and George Kohler ½× 2 = 1

4. GRAS organisms are non-pathogenic & non-toxic (1)

OR

Because they are generally regarded as safe

5. In the presence of DMSO, ice crystals do not form and cell membranes do not rupture at low temperatures ½

High concentration of serum probably contributes to cell integrity ½

SECTION B

6.

	P^{BR322}	P^{UC19}
(a)	Has 2 antibiotic resistance genes (amp^r & tet^r)	Has only amp^r gene
(b)	no lac Z gene	lac Z that codes for β -galactosidase

1 + 1=2

7. Y (1)

3' CTGTAG/AATG/T/C 5' (1)

8. (a) To sterilize the medium that would otherwise contaminate the cultures. (1)

(b) Membrane/ultra filtration (1)

9. Def: Unorganized mass of cells ½

— maintained by repeated sub culturing ½

— Plant regeneration
Preparing single cell suspension
Protoplast
Genetic transformation studies. any two ½×2 = 1

10. T ceils play a major role in rejection of foreign grafts. OKT3 is a monoclonal Ab that targets CD3 surface marker on mature T cells & removes them from circulation. (1)

11. Site directed mutegenesis ½

Descriptive form or diagram to explain steps involved 1½

12. (a) BASIC LOCAL ALIGNMENT SEARCH TOOL (1)

(b) Homology searches (1)

13. $X\mu = \frac{dx}{dt}$

$\mu = \frac{2.303\,(\log_{10} X - \log X_0)}{t - t_0}$ ½

Substituting $X = 10^{10}$

$X_0 = 10^5$

$t = 4$

½

doubling time $= \frac{0.693}{\mu}$ ½

$= \frac{0.693}{2.9} = 0.238$ hr ½

14. (a) Bt cotton is transgenic for Cry/Bt genes from bacillus thuringiensis and they make cotton plants resistant to insect pest Bollworm (1)

(b) Increased yield, reduction in pesticide use $\mu = \frac{2.303\,(10-5)}{4} = 2.90\ \text{hr}^{-1}$ (½+½)

15. (a) In a fed batch culture, the culture system is continuously and sequentially fed with fresh medium without removing the growing culture. Over a period of time the vol. of the culture goes on increasing. (1)

(b) It is better than a batch culture, because of addition of fresh medium the organism keeps on growing further. (1)

SECTION C

16. (a) Sickled RBC resist malarial infection: (1)

(b) The substitution of glutamic acid with valine in ScHb results in increase in hydrophobic interaction between the Hb molecules resulting in aggregation and ultimately leading to deformation of RBC to a sickle shape (1+1)

17. SNP's are single nucleotide polymorphisms ½

Def. DNA sequence variations which occurs when a single base is altered so that different individuals have different base at these, portions. ½

Importance: Criminology/forensic, medicine, population genetics (any two) (1+1)

18. (a) Refer to fig. 3/ppl11 (diagrammatic or descriptive form) 2½

(b) Streptomyces gresius ½

19. One can produce virus -free plants from virus - infected plants by collecting meristems. then micro propagating them in culture, as meristems are devoid of virus. (1)

No (1)

They are not virus resistant as they are derived from virus sensitive plants. (1)

20. (a) CO_2 incubators - reproduce as closely as possible the environmental condition of living cells. (constant temp./high humidity/fixed level of CO_2/ sterility). (1)

(b) Centrifuge - centrifuges are required for separating cells or their components with a gentle braking action helps prevent disruption of the separated bands of cells. (1)

(c) Inverted microscope - for visualizing cells in situ or it allows cells at the bottom of a petri plate to be visualized because the optical sys. is at the bottom with the light source at the top. (1)

21. Mass Spectrometer (1)

Determines the molecular weight of chemical compounds by separating molecular ions according to mass/ charge ratio m/z. (2)

OR

No correlation between mRNA and protein expression (1)

mRNA undergoes differential post transcriptional modifications (1)

Proteins undergo different posttranslational modification (1)

Refer to pp. 38/Fig. 15

Can consider other techniques also.

22. Indicate through diagram or in points (steps should include).

- 'Choosing cell population and extracting mRNA.
- Reverse transcribing the mRNA to get cDNA.
- Fluorescent labelling of cDNA
- Hybridization to a DNA microarray.
- Scanning the hybridized array.
- Interpretation of scanned image ½×6 = 3

23. Breeding assisted by molecular (nucleic acid) markers (1)

Type of markers used : (Any two)

*Molecular marker based on DNA polymorphism detected by DNA probes

or

amplified products of PCR eg. VNTR, RAPD

*Morphological marker based on visible character eg. color, seed.

* Biochemical marker based on detection of natural enzymes eg. isozymes. 1+1

24. Complete the table:

Step	Specific activity (units/mg)	(1)
I	200	
II	600	
III	667	
IV	1500	½×4 = 2

25. 3 major factor groups

- growth factors
- amino acids, hormones
- Salts containing ions as Ca, K etc. (1 + 1 + 1)

Section 'D'

26. They recognize a sequence & then make cuts in DNA, either within the recognized sequence/away from it. (1)

- *EcoRI Type II RE (1)
- 5'-GAATTC-3' Sequence recognized by EcoR I (1)
- Refer to Table 1 /pp45 for other enzymes.
- Bacteria produce RE as a part of their defense against entry of foreign DNA. (1)
- * By methylating a base in the sequence recognized by the RE (1)

OR

(a) A cDNA library is a collection of clones representing cDNA from a cell type/ tissue in a recombinant vector (1)

Advantage : (1) Represents expressed genes
(2) Lacks introns therefore library represents the coding sequence of gene 1+1

(b) ddNTP when incorporated into DNA they terminate chain elongation. (1)

Structure of ddCTP (dideoxy cytidine or any ddNTP) (1)

27. (a) Hydrophobic interactions
Hydrogen bonds
Van-der Waals
*Ionic ½×4 = 2

(b)

Hydrogen bonds	**Van der waals**	
Sharing of hydrogen atom with electronegative atoms stronger	weak attraction occur at close range	(1)
Partial covalent band	partial covalent bands are not involved	(1)

(c) Prions- incorrectly shaped proteins which cause normal proteins to turn diseased eg mad cow disease. ½×2 = 1

28. (a) DNA template, Taq/DNA polymerase, 2primers, dNTP ½×4 = 2

(b) Stable at 94° C temperature used for denaturation (1)

(c) detecting infections, forensics/criminology, genetic basis of disease

(any two) 1×2 = 2

OR

(a) Blue-white selection based on insertional inactivation of Lac Z gene present on the vector. (1)

Incorporation of foreign DNA fragment into this Lac Z gene will lead to inactivation of Lac Z gene resulting in prevention of blue color; white colonies are formed due to the presence of rDNA. (2)

(b) Any two of the following :
Transformation, Transfection, Electroporation, Microinjection, Biolistics, Bacteriophage 1×2 = 2