BIO-TECHNOLOGY

Time allowed : 3 hours ***Maximum Marks : 100***

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 two questions of five marks. You have to attempt only one of the choices in such questions.

(iii) Questions number 1 to 5 are very short answer questions, carrying 1 mark each.

(iv) Questions number 6 to 15 are short answer questions, carrying 2 marks each.

(v) Questions number 16 to 25 are also short answer questions, carrying 3 marks each.

(vi) Questions number 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. Farmers growing cotton find that bollworm is devouring their crop. Suggest a possible way by which genetic engineering can provide a solution to this problem. 1

2. A microbiologist involved in an antibiotic discovery programme has isolated some bacteria from hot springs in the Himalayas. However attempts of growing these bacteria under regular laboratory conditions have failed. What suggestion can you give him? 1

3. An enriched medium containing salts, glucose, proteins and vitamins was made and a commercially available animal cell line was introduced. However the cells began dying. What could have been the mistake? 1

4. In micro-propagation, axillary meristems are used as the source for raising virus free plants, why? 1

5. A given microbial species grows rapidly. Which will be higher, its specific growth rate μ or doubling time 't' ? 1

6. What is the role of Factor VIII? Why are animal cell lines required to produce it ? 2

7. Differentiate between homologous and paralogous genes. 2

8. Write the principle of Southern hybridization. 2

9. 'Whey', in ancient times, was recommended for its therapeutic role. Why? 2

10. Indicate two important properties of animal cell cultures. 2

11. What are haploids and triploids ? What are their uses in plant cell culture? 2

12. Indicate the use of the following in microbial cell culture (a) liquid nitrogen (b) agar (c) nitrosoguanidine (d) glycerol 2

13. Expand SNPs and describe their one application. 2

14. Give the principle of the blue-white screening method in rDNA technology. 2

15. In the enzyme chymotrypsin, why does Ser195 develop a negative charge on its -OH group. 2

16. Why is Agrobacterium tumefaciens referred to as a 'natural genetic engineer' ? How does this bacterium achieve this feat? 3

17. After growth in fermentor, downstream processing involves a number of techniques. Explain the principles behind (a) centrifugation (b) ultrafiltration. What is the rational behind minimizing steps involved in downstream processing? 3

18. What do you understand by the term "genomics" ? Differentiate between structural and functional genomics. 3

19. In an experiment involving cloning of a genomic DNA fragment into a vector: 3

    (a) Suggest a vector to clone this fragment, if its size is approx 300 kb.

    (b) Suggest a cloning strategy if the DNA has very uncommon restriction enzyme sites at its ends.

20. Indicate two disadvantages of vegetative propagation of plants. How does micropropagation overcome these and briefly outline the steps involved. 3

21. In a form of Alzheimers, a victim's brain tissue after an autopsy showed abnormal precipitates which appeared to be proteinaceous. Explain the modern methods which may be used to identify these abnormal proteins. 3

22. What are the 3 main features that a vector should possess and explain the role of each. 3

OR

Do you consider phage based vectors superior to plasmids ? Justify your answer.

23. The publication of 'Atlas of Protein Sequence and Structure' under the editorship of Margaret O. Dayhoff was a pioneering effort. Why? 3

24. Briefly describe the commonly used culture methods used for biomass or metabolite production. 3

25. Foot and Mouth Disease Virus (FMDV) vaccine is made by growing the virus in animal cells, harvesting the virus and inactivating it for vaccine formulations. Given the following data, calculate the weight and volume of the harvested virus from a bioreactor.

(1) Total bioreactor capacity = 1000 1 (atleast 20% space must be kept for oxygen and CO_2)

(2) No. of animal cells = 10^5/ml.

(3) No. of virus particles/animal cell = 50

Assume the virus to be a sphere with a gm molecular weight of 10^6 (1 million) and r = 1 nm. 3

26. Expand the term 'BLAST'. Discuss the steps involved in comparison of DNA sequences using this tool. 5

OR

Name any five major databases commonly used in bioinformatics. Also suggest the type of information available from each of these databases. 5

27. What is the hierarchical organization in protein structure? Indicate the nature of covalent and non-covalent forces which determine the protein structure. 5

OR

How can one use the method of aqueous - two-phase partitioning for separation of proteins? Also suggest various efforts which may be taken to, maximize protein stability during such separation. 5

28. Why have restriction enzymes been so named? Only type II enzymes are used in recombinant DNA technology, why? With an example, illustrate

(a) a blunt end cutter

(b) a sticky end cutter 5

QUESTION PAPER CODE 99

SECTION A

1. Name the bacterium toxin which is used to engineer crops resistant to bollworms. 1

2. How would you grow a bacterium in the laboratory which has been isolated from a hot spring? 1

3. Animal cells in a culture medium were placed in a regular incubator used for growing bacterial cells. Do you expect the animal cells to grow or not? 1

4. In micropropagation apical meristems are used for raising virus-free plants. Why? 1

5. A given microbial species grows slowly. Of the two - specific growth rate (μ) or doubling time (t), which one would be lower? 1

SECTION B

6. CHO animal cell line is used to express r-HuEPO. Why? What is the function of this protein? 2

7. Indicate any two Bioinformatics databases and their uses. 2

8. Write the structure of a dideoxynucleotide triphosphate and its role in DNA sequencing. 2

9. Why is 'curd' considered beneficial? 2

10. Briefly list the features of finite cell lines and continuous cell lines. 2

11. Differentiate between somaclones and gametoclones. 2

12. Indicate the use of the following in microbial cell cultures: 2

(a) Aeration, (b) Agar, (c) Antifoams, (d) Corn-steep liquor.

13. Genome analysis has the potential to identify patients with disease susceptibilities. Explain. 2

14. Highlight the principle of 'insertional inactivation'. 2

15. How does the charge -relay system operate in the enzyme Chymotrypsin? 2

SECTION C

16. How is transformation of plant tissue achieved using *Agrobacterium tumefaciens* ? Indicate the salient steps. 3

17. Outline the steps and principles involved in isolating Streptomycin, an extracellular microbial product. 3

18. Schematically depict the steps involved in Fluorescence In Situ Hybridization (FISH). 3

19. In the diagnosis of tuberculosis, the older methods depended on culturing the causative bacillus from sputum. Newer methods include PCR-based assays. With the help of a diagram explain the principle of PCR-based assay. How is it more effective than culturing methods? 3

20. Describe protoplast culture and its applications. 3

21. Thalassaemic patients produce excess alpha or beta sub-units of haemoglobin leading to impaired oxygen binding capacity by their erythrocytes. How can it be determined as to which sub-unit is produced in excess? 3

22. Describe three vectorless DNA transfer methods. 3

23. Why are sequence databases important? Name atleast three such databases and their uses. 3

24. A given recombinant protein is expressed intracellularly in E. coli. Which culturing method is best suited for obtaining maximum yield of this protein? Explain. 3

OR

Differentiate between fed-batch and continuous microbial culture. 3

25. Foot and Mouth Disease Virus (FMDV) vaccine is made by growing the virus in animal cells, breaking the cells, harvesting the virus and finally inactivating it before vaccine formulation. Based on the data given below, calculate the packed volume and weight of virus harvested: 3

(a) Total bioreactor/fermentor volume = 2000 L
(atleast 20% space must be kept for oxygen and CO_2)

(b) No. of animal cells in culture = 105/mL .

(c) No. of virus particles per animal cell = 50

(d) Molecular mass of virus = 10^6 (1 million)
(Assume virus is a sphere of radius 1 nm)

SECTION D

26. What are the advantages of whole genome sequencing projects? How is gene prediction carried out in such projects using computational tools? 5

OR

What is meant by the term "genomics"? Differentiate between structural and functional genomics. 5

27. One of the first examples of molecular disease was sickle cell anaemia. Describe the technique which was used to establish this discovery. 5

28. With a suitable diagram, explain how RFLP technique is useful for differentiating DNA sequences. 5

OR

How is a cDNA library generated and what are its uses? 5

Marking Scheme ó Biotechnology

General Instructions

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3. Questions number 1 to 5 are very short answer questions, carrying 1 mark each.
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5. Questions number 16 to 25 are also short answer questions, carrying 3 marks each.
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QUESTION PAPER CODE 99/1

EXPECTED ANSWERS/VALUE POINTS

Q1. Bt toxin gene/cry gene can be introduced into the cotton plant. 1

Q2. Maintain cultures at higher temperatures. 1

Q3. Serum/FCS an essential component of animal cell culture media must be added. 1

Q4. Generally meristems are virus free. 1

Q5. Specific growth rate 'μ' will be higher. 1

Q6. Factor VIII is a blood clotting factor. 1

Animal cell lines are required to produce large amounts/post translational modifications. 1

Q7. Homologous genes are descended from a common ancestor. 1

Paralogous genes are duplications within a species/genome. 1

Q8. DNA fragments are separated by agarose gel electrophoresis based on size and transferred to a nitrocellulose membrane. 1

The transferred sequences can be hybridised with DNA probes and specific genes/ sequences can be detected. 1

Q9. Whey causes elevation of glutathione which plays a role in detoxification of xenobiotics/oxygen intermediates/free radicals. 1

Whey is used for treatment of diseases like jaundice etc. 1

Q10. Animal cell cultures show contact inhibition/finite life span/anchorage dependence (any two). 1 + 1= 2

Q11. Haploids have single set of chromosomes/karyotype/n and triploids have three sets/ 3n. 1

Haploids are used for expression of recessive traits and triploids are used for producing sterile plants/seedless fruits. ½ + ½ = 1

Q12. Liquid nitrogen is used for low temperature storage.

Agar is used for making solid culture media.

Nitrosoguanidine is used for generating mutations.

Glycerol is a cryopreservant/prevents ice crystal formation. ½ x 4 = 2

Q13. Single Nucleotide Polymorphism. 1

SNPs used in prediction of disease/ forensic science/population genetics. 1

Q14. Genes are cloned in vectors which use ß-galactosidase activity as a marker. If enzyme activity is there, a colorless substrate x-gal is hydrolysed to a blue color; however if the ß-gal/lac z' gene is interrupted (insertional inactivation) a white colony results. 2

Q15. Asp 102, His 57 and Ser 195 lie in this order forming a charge relay; 1

The negatively charged aspartate carboxylate residue pulls the serine –OH proton through his, leaving it with a negative charge. Same can be depicted diagrammatically. 1

Q16. A.tumefaciens has a natural ability to infect plants at a wound site. 1

It has a Ti or tumour inducing plasmid. 1

T DNA is transferred into plant cell. 1

Q17. (a) Centrifugation: removes material difficult to sediment. 1

(b) Ultrafiltration: Passing broth through filters to remove cells/debris for obtaining clear filtrate. 1

(c) Minimising steps: Cost effective/ less denaturation of protein higher yield. 1

Q18. Genomics includes mapping, sequencing and analysing genomes of all species. 1

Structural genomics: Assembly/ organisation/ management of DNA sequences. 1

Functional genomics: Biological function/interaction of genes. 1

Q19. (a) BAC/YAC vector can be used. 1

(b) Vector containing a MCS(multiple cloning site)/polylinker with that restriction site can be used/ Diagram on pg. 55 can be given. 2

Q20. Disadvantages of vegetative propagation- labour intensive/low productivity/seasonal (any two). ½ + ½ = 1

Micropropagation is rapid/any other application. 1

Steps- initiation, shoot formation, root formation and transplantation. 1

Q21. Autopsy samples from normal and diseased brain taken as source sample. ½

Samples subjected to 2D gel electrophoresis, pg.16 & 17. 2

Comparison of stained gels to identify abnormal protein. ½

Q22. 1. Origin of replication, 2. Selection markers, 3. Restriction sites.

Briefly write about their importance. 1 x 3= 3

Or

Phage based vectors better due to natural infectivity and larger insert capacity. 2

M13 phage can provide single strand DNA required for sequencing. 1

Q23. Macromolecular sequences first compiled in this Atlas. 1

Development of computer methods for comparison of protein sequences. 1

Detection of various features from sequences like duplications, evolutionary histories, alignments. 1

Q24. Description of any two- Batch culture, fed batch culture, continuous culture.pg.105. 1½+1½

Q25. Culture volume = 80/100 x 1000 = 800L ½

No. of animal cells = 10^5x 1000 x 800 = 8 x10^{10}L ½

No. of virus particles = 50 x 8x 10^{10}= 4 x 10^{12}

10^6 g (M.wt) has 6.023 x 10^{23} virus.

Hence 4x10^{12} particles have wt. = 4x10^{12}x10^6/ 6.023 x 10^{23} = 0.667 x 10^{-5}g 1

Volume of one virus particle = 4/3πr3= 4/3 x 22/7 x 1^3nm^3

Volume of 4x 10^{12} particles = 1.67 x 10^{13} nm^3 1

Q26. Basic Local Alignment Search Tool. ½

Steps b to d as on pg. 93 1½ +1½ +1½

Or

EMBL, SWISS-PROT, PDB, Ribosomal RNA database, PALI with details pg.94. 1x5

Q27. Primary, secondary, tertiary and quaternary structure with details, pg. 9. 2

Covalent- disulfide bonds. 1

Non-covalent- hydrogen, Van der Waals, hydrophobic, ionic. 2

Or

Aqueous two phase partition with diagram on pg.22. 2

Stabilising steps 1-5 (any three)pg. 23. 3

Q28. Restrict growth of phages in bacteria. 1

Type II restriction enzymes recognise and cut within sequence. 1

Blunt end cutter- Sma I/any other with diagram, pg.46. 1½

Sticky end cutter- Eco RI/any other with diagram, pg.46. 1½

QUESTION PAPER CODE 99

EXPECTED ANSWERS/VALUE POINTS

SECTION A

Q1. Bt /cry gene product/CRY 1

Q2. Grow culture at higher temperature. 1

Q3. No, animal cells need a CO_2 incubator. 1

Q4. These are virus free. 1

Q5. Specific growth rate 'μ' is lower. 1

Section B

Q6. CHO cell lines provide appropriate post-translational modifications. 1

EPO, stimulates erythrocyte proliferation. 1

Q7. EMBL/EBI/NCBI/PIR (any two) and their uses. Pg. 87/94 1 + 1

Q8. Structure as on pg.64. 1

Prevents addition of new nucleotide on 3' end terminating DNA synthesis. 1

Q9. Contains beneficial bacteria(probiotics) to manage intestinal infections/rich source of whey which elevates glutathione. 2

Q10. Finite cell lines have limited life span/contact inhibition/anchorage dependence,(any two Continuous cell lines have limited life span/no contact inhibition/anchorage independant/doubling time low etc.(any two) pg. 146. 1 + 1

Q11. Somaclones are from somatic tissue; gametoclones are from pollen or egg. 1 + 1

Q12. (a) Improves oxygen availability for growth.

(b) Used for solidifying media.

(c) Prevents foaming.

(d) Source of Nitrogen. ½ x 4 = 2

Q13. SNPs/sequences are identified for probable association with disease and other susceptibilities. 2

Q14. Sequence/insert cloned into vectors within genes of antibiotic resistance/ß- galactosidase enzyme leading to loss of function 2

Q15. In the 3D structure of chymotrypsin asp 102, his 57 and ser 195 are close together allowing a charge relay to form; the negative charge of asp causes a negative charge on ser through his/diagram can also be drawn to indicate the same. 2

Section C

Q16. Ti plasmid engineered to carry the relevant gene is used to transform *A.tumefaciens* which then infects the plant/plant cell using its natural ability to be transferred. Pg.127 for diagram/details. 3

Q17. Steps- fermentation,filtration,clear broth subject to liquid extraction/chromatography, concentration,purification and crystallisation.pg. 111. 3

Q18. Flourescent nucleotides introduced at selective genes/sequences in chromosomes by nick translation and analysis. Details on pg.83. 3

Q19. Amplification of gene sequences specific to M.tb using diagram,pg. 60. Technique is more rapid and sensitive. 1+2

Q20. Generate protoplasts by removing cell wall enzymatically, pg.122. 1

Uses- metabolic studies/ fusion of somatic cells/ making cybrids/ genetic manipulation (any two). 2

Q21. Normal and thallasaemic erythrocytes obtained and their lysates analysed. ½

Protein fingerprinting/2-D gel/MALDI-TOF/SDS-PAGE can identify if α or ß chain is absent. Describe any one technique. 2½

Q22. Chemical mediated/microinjection/electroporation/particle gun (any three)pg.128. 3

Q23. EMBL, SWISS-PROT, PDB, Ribosomal RNA database, PALI (any three) with details pg.94. 1 x 3

Q24. Fed batch culture more useful. Details on pg. 105. 3

Or

	Fed batch culture	Continuous culture
1.	Nutrients added without removal of culture.	Nutrients added with removal of culture.
2.	Volume increases.	Volume constant.
3.	Used for high cell density. biomass/metabolite production.	Used for 1 x 3

Q25. Culture volume = 80/100 x 2000 = 1600L ½

No. of animal cells = 10^5x 1000 x 1600 = 1.6 x10^{11}L ½

No. of virus particles = 50 x 1.6 x 1011= 8 x 10^{12}

10^6 g (M.wt) has 6.023 x 10^{23} virus (Avagadro no.).

Hence 8x10^{12} particles have wt. = 8x10^{12}x10^6/ 6.023 x 10^{23} = 1.334 x 10^{-5}g 1

Volume of one virus particle = 4/3πr^3= 4/3 x 22/7 x $1^3$$nm^3$

Volume of 8 x 10^{12} = 3.34 x 10^{13} nm^3 1

Section D

Q26. Provides basis for discovery of genes.

Serves as inventory of all genes.

Establishes relationship between genes.

Provides a set of tools for future experimentation.

Acts as index to draw and organise all genetic information.

Acts as an archive. Any four.pg.78. 1 x 4

Gene prediction can be done using various algorithms. 1

Or

	Scientific discipline of mapping, sequencing and analysing genomes.	1
	Structural genomics: Assembly, organisation and management of DNA sequences.	2
	Functional genomics: Biological function of genes and their interaction.	2
Q27.	Protein fingerprinting technique. Steps with diagram pg.15- 16	4
	Identify peptide different from normal and sequence.	1
Q28.	Restriction fragment length polymorphism.	1
	Technique details, pg. 48.	3
	Uses- forensic science/parentage dispute.	1

Or

	cDNA is complementary to mRNA, made using reverse transcriptase.	1
	Details of technique on pgs. 56- 57.	3
	Useful for obtaining protein encoding genes with no introns.	1