BIOTECHNOLOGY

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. Question paper contains four sections - A, B, C and D.

(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. Why is humulin considered better than pig insulin for the treatment of diabetes? 1

2. If a researcher began with a sample that contained 5 copies of double stranded DNA, how many copies would he be able to get after 20 cycles of PCR ? 1

3. What are interferons? 1

4. In isolating recombinant insulin from a culture of *E.coli*, the cells were filtered and the filterate was subjected to a purification protocol. However no insulin was obtained. Why? 1

5. Why is erythropoietin (EPO) included in the list of banned substances for sports-men? 1

SECTION - B

6. In a batch culture of *E.coli*, specific growth rates of the microbial cells will be maximum at which phase of growth and why? 1+1 = 2

7. Compared to a conventional plasmid, what additional sequences are required in a YAC vector so that it can behave as an artificial chromosome? 2

8. Why is it useful to search a database to identify newly determined DNA sequence? Give two reasons. 2

9. Why is foaming caused during fermentation process? How can this be harmful to the process? 1 + 1 = 2

10. What is a transgenic plant? Enlist two examples of transgenic plants with beneficial traits. 2

11. Patients who are administered monoclonal antibodies against CD3 can accept renal allograft, why? 2

12. Listed below are four different single strands of DNA. Which of these in their double stranded form, would you expect to be cleaved by a restriction endo-nuclease, and why?

    (a) GCCTCATTCGAAGCCTGA

    (b) ACTCCAAGCTTCACTCCG

    (c) CTCGCCAGACTCGTCGCA

    (d) ACTCCACTCCCGACTCCA 2

13. (a) Expand 'BLAST'.

    (b) When aligning two or more genetic sequences, it is sometimes necessary to insert gaps, why? 2

14. How are novel genes introduced into plants using Ti plasmid of Agrobacterium ? Enlist major steps. ½ x 4 = 2

15. A soil microorganism produces a novel metabolite in nanomolar (nM) concentration. Suggest any two ways to increase its production. 2

SECTION - C

16. What is 'Molecular Pharming'? Why is it more advantageous compared to production in a bacterial system? Give any four reasons. 1+½ x 4 = 3

17. DNA microarray permits an investigator to monitor simultaneously, the level of mRNA production from every gene in an eukaryotic organism.

    (i) Why might such an analysis not give an accurate estimate of the level of protein expressed in an organism?

    (ii) Which alternative technique will be better suited for the above mentioned analysis? 3

18. List three *differences* between a batch and a continuous culture. 3

19. What are the potential risks (any three) and benefits (any three) of GM crops? 3

20. Why is it difficult to culture animal cells as compared to plant or microbial cells? Why is it essential to supplement animal cell culture media with serum?

OR

Why are animal cells grown in CO_2 incubators and not in regular incubators? Give three reasons. 3

21. (i) What are essential amino acids?

    (ii) Athletes are recommended to consume Branched Chain Amino Acids (BCAA) before and after exercise. How does this practice benefit them? 3

22. What is a recombinant vector? How is it constructed? 3

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

24. Embryonic stem (ES) cells could potentially be used to treat a variety of diseases

associated with cell and tissue damage. Defend this statement by giving three examples of ES therapeutics. 3

25. Study the following purification table and answer the questions that follow:

Steps	Procedure	Total protein (mg)	Activity (units)
Step 1	Crude extract	15,000	150,000
Step 2	Salt fractionation	4,000	138,000
Step 3	Ion exchange chromatography	1,500	115,500
Step 4	Size exclusion chromatography	68.8	75,000
Step 5	Affinity chromatography	1.75	52,000

(i) Which step in the purification is most effective, and why?

(ii) Which of the procedure is least effective and why? 3

SECTION - D

26. (a) What do you understand by 'SNPs'? Suggest any two applications.

(b) Name any two databases important in bioinformatics. Mention the type of information which may be obtained form these databases. 3 + 2 = 5

27. (i) What is meant by proteomics ?

(ii) Name three important types of proteomics.

(iii) Why is the proteome of a given species larger than its genome? Give two reasons. 1½+1½+2 = 5

OR

Name the technique developed by O'Farrel. Schematically depict the key steps in the separation of proteins using this technique. Highlight the basis of separation at each step.

28. (i) Schematically illustrate the technique of 'site-directed mutagenesis'.

(ii) What physical and chemical properties of naturally occurring enzymes might be useful to change by site directed mutagenesis?

OR

Explain the principle and steps involved in the Sanger's method of DNA sequencing. 5

QUESTION PAPER CODE 99
SECTION A

1. Protein chemists prefer to monitor absorbance of protein fractions eluting from a chromatographic column at 280 nm. Why? 1

2. If a researcher began with a sample that contained three copies of double stranded DNA, how many copies would he be able to generate after 27 cycles of PCR ? 1

3. What are monoclonal antibodies? 1

4. Recombinant human insulin cannot be obtained from the culture filtrate of *E.coli.* Why? 1

5. Why are long distance runners disqualified if they test positive for high amounts of erythropoietin (EPO) ? 1

SECTION - B

6. Most media that are used for culturing microbes within laboratories are not used for large scale cultivation. Why ? 2

7. If you wanted to express a eukaryotic protein in bacterial cells, would you clone genomic DNA or cDNA into the expression vector? Justify your choice. 2

8. What are homologous sequences? How can you access information on homologous genes/sequences? 2

9. Why is aeration important for microbial growth? How can proper aeration be achieved in microbial cultures grown under laboratory conditions ? 2

10. Differentiate between primary and secondary metabolites. Name two secondary metabolites obtained through tissue culture and their application in medicine. 2

11. Monoclonal antibody against CD3 is an effective therapeutic agent in overcoming renal allograft rejection. Why ? 2

12. Listed below are four different single strands of DNA. Which of these in their double stranded form would you expect to be cleaved by a restriction endonuclease and why? 2

    (a) ACTCCAGAATTCACTCCG

    (b) ACTCCACTCCCGACTCCG

    (c) GCCTCATTCGAAGCCTGA

    (d) GAGCGGTTTATCTGAGCAG

13. (a) Expand 'EMBL'.

    (b) Why is it necessary to insert gaps when aligning two or more genetic sequences? 2

14. Which plant part(s) would be best suited for expressing antigens and why? 2

15. A fungal extract has anti-cancer potential and it has shown positive results in clinical trials against childhood leukemia. However, the active compound is present in very low concentration. Suggest any two ways to increase its production. 2

SECTION - C

16. What is 'Molecular Pharming' ? Suggest any four advantages of expressing transgenic proteins in milk. 3

17. Suggest any four methods used for introduction of recombinant DNA into host cells. Also explain the method of insertional inactivation used for identification of the recombinants. 2+1

18. What is a 'fed-batch' culture? What are its benefits in microbial technology ? 3

19. What are the major constraints in accepting transgenic crops? 3

20. (i) Briefly explain what is meant by 'contact inhibition'.

(ii) What is the difference between a defined and a serum-supplemented medium? 3

OR

(i) Differentiate between anchorage-dependent and anchorage-independent cells.

(ii) How is damage to cells prevented during cryopreservation ?

21. (i) Give any two applications of proteolytic enzymes.

(ii) How does the consumption of branched chain amino acids (BCAA) help athletes in enhancing their performance? 3

22. What information can be obtained from genome sequencing projects? 3

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

24. How could embryonic stem (ES) cells potentially be used for treatment of diseases associated with tissue damage? Give two examples. 1+2 = 3

25. Study the following enzyme purification table and answer the questions that follow:

Steps	Procedure	Total protein (mg)	Activity (units)
Step 1	Crude extract	10,000	1,00,000
Step 2	Salt fractionation	3,000	96,000
Step 3	Ion exchange chromatography	400	80,000
Step 4	Size exclusion chromatography	300	60,000
Step 5	Affinity chromatography	3	45,000

(a) Which step in the purification is most effective and why?

(b) Which of the procedures is least effective and why? 3

SECTION - D

26. (i) What are the essential features of a vector?

(ii) What is the role of cos sites in phage λ ?

(iii) What is the role of DNA ligase and alkaline phosphatase in recombinant DNA technology ? 5

27. (i) What is isoelectric focussing?

(ii) Name some of the important branches of proteomics.

(iii) Why is the study of proteome relevant in the age of genomics ? 5

OR

What is the principle of protein fingerprinting? Enlist major steps for performing this technique. Suggest one application of this technique for detection of human disease.

28. Expand NCBI. What are the possible uses (any two) of databases available in NCBI ? How can tools available in these databases be used to retrieve and compare genetic information? 5

OR

Gene prediction for a given genome using bioinformatic tools may be different from the actual number of genes identified by experimental methods. Why is it so ? Do you think there is always a correlation between the complexity of the organism and total number of genes present in its genome? Justify with suitable example.

Marking Scheme — Biotechnology

General Instructions :

The Marking Scheme and mechanics of marking

1. All awarded marks are to be written in the left hand margin at the end of the question or its part.
2. Place a tick (✓) in red directly on the key/operative term or idea provided it is in correct context. Place "Half-tick" ½ wherever there is ½ mark in the marking scheme. (Do not place tick indiscriminately just to show that you have read the answer).
3. If no marks are awarded to any part or question put a cross (×) at incorrect value portion and mark it zero (in words only).
4. Add up ticks or the half ticks for a part of the question, do the calculation if any, and write the part total or the question total in the left hand margin.
5. Add part totals of the question and write the question total at the end. Count all the ticks for the entire question as a recheck and draw a circle around the question total to confirm correct addition.
6. If parts have been attempted at different places do the totalling at the end of the part attempted last.
7. If any extra part is attempted or any question is reattempted, score out the last one and write "extra".
8. In questions where only a certain number of items are asked evaluate only that many numbers in sequence as is asked ignoring all the extra ones even if otherwise correct.
9. Transcribe the marks on the cover page. Add up question totals. Recheck the script total by adding up circled marks in the script.
10. Some of the questions may relate to higher order thinking ability. These questions will be indicated to you separately by a star mark. These questions are to be evaluated carefully and the students' understanding / analytical ability may be judged.
11. The Head-Examiners have to go through the first five answer-scripts evaluated by each evaluator to ensure that the evaluation has been carried out as per the instruction given in the marking scheme. The remaining answer scripts meant for evaluation shall be given only after ensuring that there is no significant variation in the marking of individual evaluators.

QUESTION PAPER CODE 99/1

EXPECTED ANSWERS/VALUE POINTS

SECTION A

Q1. Humulin acts faster/Incidence of allergic reaction is reduced. 1

Q2. $(5 \times 2)^{20} = 10^{20}$ 1

Q3. Interferons are proteins secreted by virally infected cells and interfere with viral propagation. 1

Q4. Recombinant insulin is expressed intracellularily, hence no insulin is in the culture filtrate. 1

Q5. EPO enhances performance of athletes because it stimulates RBC production, which increases oxygen carrying capacity, aerobic metabolism; hence it is banned. 1

Section- B

Q6. Specific growth rate is maximum during exponential/ log phase. 1

Availability of nutrients allows cells to divide rapidly till stationary phase. 1

Q7. YACs contain teleomeric and centromeric sequences which enable it to behave as an artificial chromosome. 1 + 1=2

Q8. To identify genes, their functions, regulatory sequences and to infer phylogenetic relationships (any two). 1 + 1= 2

Q9. Foaming is caused by proteins/components of media/metabolites produced by microbes (any one). 1

Foaming can denature proteins/cut oxygen supply/microbes trapped in foam cannot grow (anyone) 1

Q10. A transgenic plant has DNA/genes from another organism introduced through recombinant DNA technology. 1

Any two examples- Bt cotton, Flavr Savr tomatoes, Golden rice, edible vaccines. ½+½ = 1

Q11. T cells playa major role in graft rejection.

OKT-3 targets CD3 surface marker on T cells removing them from circulation and hence graft is not rejected. 1 + 1= 2

Q12. Options (a) GCCTCATTCGAAGCCTGA

and (b) ACTCCAAGCTTCACTCCG ½+½ =1

Restriction enzymes recognise palindromic sequences. 1

Q13. BLAST: Basic Local Alignment Search Tool. 2

Gaps are inserted to align sequences of unequal length but sequence similarities.

Q14. Use leaf discs/embryonic callus; Infect with recombinant disarmed Ti plasmid; co-cultivation to facilitate transfer; selection and regeneration medium; induce rooting; hardening and transfer to soil. 2

Q15. Strain improvement techniques; classical genetic techniques/mutant selection; genetic engineering. 1+1=2

Section - C

Q16. To create transgenic animals by direct microinjection of DNA into ova or stem cells and produce insulin and other proteins in milk on a commercial scale. 1

Any four advantages over using bacteria- large amounts of source, functionally active proteins being produced in mammals, ease of collecting milk, ease of production, moderate capital investment. ½ x 4 = 2

Q17. (i) Levels of mRNA are not necessarily correlated with protein production. 1½

(ii) 2-D gel electrophoresis/proteomics can assess the total proteins actually expressed. 1½

Q18.

	Batch culture	Continuous culture
1.	Closed culture system	open culture system
2.	Has limited amount of nutrients	nutrients are replenished

3.	Organism shows normal growth kinetics	cells are grown for extended time	
4.	Organisms are exposed to continually changing environment.	chemical environment is constant	
		Any three	1+1+1=3

Q19. Risks, any three from pg. 136. 1½

Benefits, any three from, pg.128-134. 1½

Q20. Animal cells have complex nutritional requirements, need CO_2 incubators, proper humidity conditions and grow only for limited generations. 1½

Serum has essential growth factors, hormones and other components that support the growth of many cell types. 1½

OR

CO_2 incubators help to maintain :

Fixed levels of CO_2 and therefore pH; high humidity to prevent desiccation, maintain correct osmolarity and constant temperature. 1 x 3=3

Q21. (i) Essential amino acids cannot be synthesised in the body and have to be obtained from the diet. 1

(ii) BCAA are important for muscle growth. Their intake prevents the breakdown of muscle proteins during exercise. They can be processed to yield energy. 2

Q22. Vector containing an insert is a recombinant vector. 1

Vector and fragment containing gene are isolated and cut with same restriction enzyme; joined with DNA ligase to make recombinant vector/Fig. on Pg.55. 2

Q23. Micropropagation using meristems.

No, these are not virus resistant.

Because meristems are virus-free but do not have resistance genes. 1+1+1=3

Q24. ES cells have self renewal capacity and differentiating capability and therefore can be used to treat burn victims; repair of joints damaged by injury or arthritis; repair or replacement of liver etc. (any three) 1+1+1=3

Q25. Step 5 most effective because of maximum increase in specific activity. ½ + 1 = 1½

Step 3 least effective because specific activity decreases from previous step. ½ + 1 = 1½

(Specific activity calculations can be allotted ½ mark for each part.)

Section-D

Q26. SNPs are single nucleotide polymorphisms. Applications- disease predictions and population genetics etc. on pg. 80-81 (any two) 1 + 2 =3

Any two databases listed on pg.94- EMBL/SWISS-PROT /PDB/Ribosomal RNA database/PALI database and their use. 2

Q27. (i) The entire protein complement of a cell/tissue/organism. 1½

(ii) Structural proteomics; functional proteomics; expression proteomics 1½

(iii) Proteins are more than genes due to alternate splicing/overlapping genes/post transcriptional and post translational modifications. 2

OR

2-D gel electrophoresis. First dimension by isoelectric focussing and include principle and second dimension by SDS-PAGE with principle. Details/ diagram on pg.16&17. 1+2+2=5

Q28. (i) Diagram on pg.68, fig.14. 3

(ii) Thermal stability; resistance to denaturation by organic solvents and extremes of pH; improving catalytic activity and modifying specificity. 2

OR

Principle and details on pg. 64-66 (fig.11 & 12).

Include 4 sequencing mixtures, each containing DNA single strand, primer, DNA polymerase, NTPs and ddTTP/ddATP/ddGTP/ddCTP. 2

The chain termination achieved due to the presence of dideoxy nucleotide triphosphate should be explained. 2

Reading of the sequence from the autoradiogram should be indicated. 1

QUESTION PAPER CODE 99

EXPECTED ANSWERS/VALUE POINTS

Section-A

Q1. Non-destructive, faster and simple method ½ x 2 = 1

Q2. $(3 \times 2)^{27} = 4 \times 10^{s}$ 1

Q3. Monoclonal antibodies are epitope specific. 1

Q4. Recombinant insulin is intracellular; culture filtrate has no hormone. 1

Q5. EPO is a performance enhancing hormone and hence banned. It stimulates RBC production increasing oxygen carrying capacity. 1

Section-B

Q6. For large scale culturing media components should be economical, consistent quality, available all round the year and locally available (any two), 2

Q7. cDNA. Bacterial systems cannot splice eukaryotic gene introns. 1+1= 2

Q8. Homologous sequences are derived from the same ancestral gene.

Database retrieval tools- LOCUS links can be used. 1 + 1= 2

Q9. For efficient oxygen transfer, cell growth and mixing (any two).

Shakers / baffle flasks. 1 + 1= 2

Q10. Primary metabolites are required for basic metabolism. Secondary metabolites are not essential.

Any two examples from Table 1 on pg. 125. 1+1=2

Q11. T cells playa major role in graft rejection.

OKT-3 targets CD3 surface marker on T cells removing them from circulation and hence graft is not rejected. 1+1 = 2

Q12. Options (a) ACTCCAGAATTCACTCCG

(c) GCCTCA TTCGAAGCCTGA

Restriction enzymes recognise palindromic sequences. 1 + 1= 2

Q13. European Molecular Biology Laboratory.

Gaps are inserted when comparing sequences which are similar but of unequal length. 1 + 1= 2

Q14. Plant parts which are eaten raw such as tomatoes, banana etc.

Easy painless delivery system, low cost and no storage problems(any two). 1 + 1 = 2

Q15. Strain improvement techniques- Classical genetic techniques / mutant selection and genetic engineering techniques. 1 + 1= 2

Section - C

Q16. To create transgenic animals by direct microinjection of DNA into ova or stem cells and produce insulin and other proteins in milk on a commercial scale. 1

Advantages- high production capacity, ease of collection of source material, low operational cost/capital and ease of production. ½ x 4= 3

Q17. Any four- transformation, transfection, electroporation, microinjection, biolistics and phage based vectors. ½ x 4= 2

Any one method of insertional inactivation- loss of antibiotic resistance/ blue- white selection method as discussed on pgs. 58 & 59. 1

Q18. A fed batch culture is continuously or sequentially fed with fresh medium without removing the growing culture. 1

Benefits-increased production of intracellular metabolites; high cell density can be achieved. 2

Q19. Transgenic crops may cause allergies; they may affect biodiversity; allow horizontal transfer of antibiotic resistance genes; change evolutionary patterns. (any three from pg.136) 1x3 = 3

Q20. (i) The phenomenon which prevents cells from growing beyond confluence. 1

(ii) A defined medium has known chemicals, of fixed composition and can support growth of selected cells.

Serum is an essential component of animal cell culture media and is a source of growth factors and hormones. 1+1 = 2

OR

(i) Anchorage dependent cells grow as adherent cells whereas anchorage-independent cells grow as suspension cultures. 1½

(ii) By adding glycerol/DMSO/serum which prevent formation of ice crystals. 1½

Q21. (i) Any two applications- eg. - dissolving clots, food and beverage industry, soap/detergent industry, leather and textile industry etc. ½ + ½ = 1

(ii) BCAA are important for muscle building; These prevent the breakdown of muscle protein during exercise; They can be processed to provide energy (any two). 1 + 1 = 2

Q22. Any three points from pg.78- basis of discovery of all genes, relationship between genes, tools for further experimentation, an index to draw and organise information, serves as an archive for the future. 1x3 = 3

Q23. Micropropagation using meristems.

No, these are not virus resistant.

Because meristems are virus-free but do not have resistance genes. 1+1+1=3

Q24. ES cells have the capacity for self-renewal and differentiation. 1

Treatment of burn victims; repair of joints; replacement of liver etc. (any two). 1 + 1 = 2

Q25. (a) Step 5 most effective- maximum increase of specific activity. ½+1 = 1½

(b) Step 4 least effective- no increase in specific activity. ½+1 = 1½

Section-D

Q26. (i) Essential features- origin of replication, selectable marker, restriction sites (any two). 2

(ii) cos sites important for packaging DNA into phage heads.

(iii) DNA ligase-joins cut DNA strands (recombinant DNA); alkaline phosphatase prevents self ligation of vector. 1 + 1 = 2

Q27. (i) Separation of proteins based on their charge/isoelectric pH. 1

(ii) structural proteomics, functional proteomics, expression proteomics, proteome mining. (any two). 1 + 1= 2

(iii) Proteins responsible for phenotype, proteins more in number than genes due to alternate splicing etc., proteome is dynamic, quantitative differences in protein expression etc. pg.37 (any two). 1 + 1= 2

OR

The principle of protein fingerprinting is to generate peptide maps after proteolytic cleavage and separation of the peptides. 1

Major steps- trypsin cleavage, electrophoresis, paper chromatography and visualisation. 3

Application- detection of sickle cell haemoglobin/ sickle cell anaemia. 1

Q28. National Centre for Biotechnology Information. 1

Uses- Comparison of genomes; Finding homology of unknown gene using BLAST. 2

Any two from pg. 92-93 2

OR

Proteins are more than genes due to alternate splicing/overlapping genes/post transcriptional and post translational modifications. 2

No correlation necessarily between complexity of organism and total number of genes. 1

Any example with justification as on pgs. 79 & 80. 1 + 1= 2