जैव प्रौद्योगिकी
Biotechnology

कक्षा / Class XII

2025-26

विद्यार्थी सहायक सामग्री
Student Support Material

केन्द्रीय विद्यालय संगठन~Kendriya Vidyalaya Sangathan

संदेश

विद्यालयी शिक्षा में शैक्षिक उत्कृष्टता प्राप्त करना एवं नवाचार द्वारा उच्च - नवीन मानक स्थापित करना केन्द्रीय विद्यालय संगठन की नियमित कार्यप्रणाली का अविभाज्य अंग है। राष्ट्रीय शिक्षा नीति 2020 एवं पी. एम. श्री विद्यालयों के निर्देशों का पालन करते हुए गतिविधि आधारित पठन-पाठन, अनुभवजन्य शिक्षण एवं कौशल विकास को समाहित कर, अपने विद्यालयों को हमने ज्ञान एवं खोज की अद्भुत प्रयोगशाला बना दिया है। माध्यमिक स्तर तक पहुँच कर हमारे विद्यार्थी सैद्धांतिक समझ के साथ-साथ, रचनात्मक - विशेषणात्मक एवं आलोचनात्मक चिंतन भी विकसित कर लेते हैं। यही कारण है कि वह बोर्ड कक्षाओं के दौरान विभिन्न प्रकार के मूल्यांकनों के लिए सहजता से तैयार रहते हैं। उनकी इस यात्रा में हमारा सतत योगदान एवं सहयोग आवश्यक है - केन्द्रीय विद्यालय संगठन के पांचों आंचलिक शिक्षा एवं प्रशिक्षण संस्थान द्वारा संकलित यह विद्यार्थी सहायक-सामग्री इसी दिशा में एक आवश्यक कदम है । यह सहायक सामाग्री कक्षा 9 से 12 के विद्यार्थियों के लिए सभी महत्वपूर्ण विषयों पर तैयार की गयी है। केन्द्रीय विद्यालय संगठन की विद्यार्थी सहायक- सामग्री अपनी गुणवत्ता एवं परीक्षा संबंधी - सामग्री संकलन की विशेषज्ञता के लिए जानी जाती है और शिक्षा से जुड़े विभिन्न मंचों पर इसकी सराहना होती रही है। मुझे विश्वास है कि यह सहायक सामग्री विद्यार्थियों की सहयोगी बनकर निरंतर मार्गदर्शन करते हुए उन्हें सफलता के लक्ष्य तक पहुँचाएगी।

शुभाकांक्षा सहित।

निधि पांडे

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Contents

BIOTECHNOLOGY

Subject Code - 045

Class XII (2025-26)

COURSE STRUCTURE

Time: 3 hours **Max. Marks: 70+30**

Units		Marks
Unit V	Protein and Gene Manipulation	40
Unit VI	Cell Culture and Genetic Manipulation	30
	Practicals	30
	Total	**100**

Theory

Time: 3 hours. **Max. Marks: 70**

Unit-V Protein and Gene Manipulation **40 Marks**

Chapter-1: Recombinant DNA Technology

Introduction, Tool of Recombinant DNA technology, making rDNA molecule, Introduction of recombinant DNA into host cells, Identification of recombinants, Polymerase Chain Reaction (PCR), DNA Sequencing.

Chapter-2: Protein Structure and Engineering

Introduction to the world of proteins, Structure-function Relationship in proteins, Characterization of proteins, Protein based products, Designing proteins (Protein Engineering)

Chapter-3: Genomics, Proteomics and Bioinformatics

Gene prediction and counting, Genome similarity, SNPs and Comparative genomics, Functional genomics, Proteomics, Information sources, Analysis using bioinformatics tools.

Unit-VI Cell Culture and Genetic Manipulation **30 Marks**

Chapter-1: Microbial Cell Culture and its Applications

Introduction, Microbial nutrition and culture techniques, Measurement and kinetics of microbial growth, Isolation of microbial products, Strain isolation and improvement, Applications of microbial culture technology.

Chapter -2: Plant Cell Culture and Applications

Introduction, Cell and tissue culture techniques, Applications of cell and tissue culture, Transgenic plants with beneficial traits, Biosafety of transgenic plants

Chapter-3: Animal Cell Culture and Applications

Introduction, Animal cell culture techniques, Applications of animal cell culture, Stem cell technology.

PRACTICALS **30 Marks**

Note: Every student will be required to do the following experiments during the academic session.

1. Use of special equipment in biotechnology experiments
2. Isolation of bacterial plasmid DNA
3. Detection of DNA by gel electrophoresis
4. Estimation of DNA by UV spectroscopy
5. Isolation of bacteria from curd & staining of bacteria
6. Cell viability assay using Evan's blue dye exclusion method
7. Data retrieval and database search using internet site NCBI and download a DNA and protein sequence from internet, analyze it and comment on it
8. Reading of a DNA sequencing gel to arrive at the sequence
9. Project work

Scheme of Evaluation

Time: 3 hours **Max. Marks 30**

The scheme of evaluation at the end of the session will be as under:

A	Two experiments	6+6 (only one computer based practical)
	Practical record	04
	Viva on Practical	04
B	Project work	
	Write up	05
	Viva on project	05
	Total	**30**

Note: - More emphasis should be given on hands on work in projects.

Prescribed Books:

1. **A TextBook of Biotechnology -** Class XI : Published by CBSE, New Delhi
2. **As reference- Biotechnology -** Class XI : Published by NCERT, New Delhi
3. **A Laboratory Manual of Biotechnology -** Class XI : Published by CBSE, New Delhi
4. **A TextBook of Biotechnology -** Class XII : Published by CBSE, New Delhi
5. **A Laboratory Manual of Biotechnology -** Class XII : Published by CBSE, New Delhi

Assessment Areas

Theory

Time: 3 hrs. **Max. Marks: 70 Marks**

Competencies	
Demonstrate, Knowledge and Understanding	50%
Application of Knowledge / Concepts	30%
Analyse, Evaluate and Create	20%

Note:

- Typology of questions: VSA including MCQs, Assertion – Reasoning type questions; SA; LA-I; LA-II; Source-based/ Case-based/ Passage-based/ Integrated assessment questions.
- An internal choice of approximately 33% would be provided.

Suggestive verbs for various competencies

- **Demonstrate, Knowledge and Understanding**

State, name, list, identify, define, suggest, describe, outline, summarize, etc.

- **Application of Knowledge/Concepts**

Calculate, illustrate, show, adapt, explain, distinguish, etc.

- **Analyze, Evaluate and Create**

Interpret, analyse, compare, contrast, examine, evaluate, discuss, construct, etc.

UNIT- 5

Protein and Gene Manipulation

Chapter 1: Recombinant DNA Technology

Recombinant DNA Technology- method used to combine DNA from two different sources to create a new set of genes in a cell or organism that can be used for various applications.

Purpose of RDT

a. Cloning of DNA (making a huge number of copies of a fragment).

b. Production of proteins by gene expression in host cells

Basic Steps of RDT

a. Isolation of DNA fragments (insert).

b. Generation of recombinant DNA (rDNA) [Vector + insert].

c. Transformation of host cell (transfer of rDNA into host cell).

d. Selection of host cells containing rDNA.

Tools of RDT

a. Enzymes – Restriction Endonuclease, DNA Ligase, Alkaline phosphatase.

b. Vectors- carrier DNA molecules.

c. Host cells

Restriction Endonuclease-

Discovered by W. Arber, H Smith and D Nathans in bacteria.

Bacterial Restriction- Modification system is a defense mechanism against viral/ phage DNA.

Restriction- Modification system contains-

a. Restriction Endonuclease- enzymes that recognize a specific DNA sequence and digests the DNA fragment. These enzymes restrict the propagation of viral DNA, hence called restriction endonuclease.

b. Methylase- enzymes that add methyl group to the bases in the recognition site and protects the host DNA from the action of restriction endonuclease.

Types of Restriction Endonuclease

Type I, Type II, Type III and Type IV Restriction endonucleases are present in bacteria.

Type II Restriction Endonuclease are used for RDT

Type II enzymes recognize a specific 4 to 8 bp long **palindromic sequence** of DNA called **restriction sites**. Palindromic sequences are sequences that read the same in both strands of DNA when read in the 5' to 3' direction.

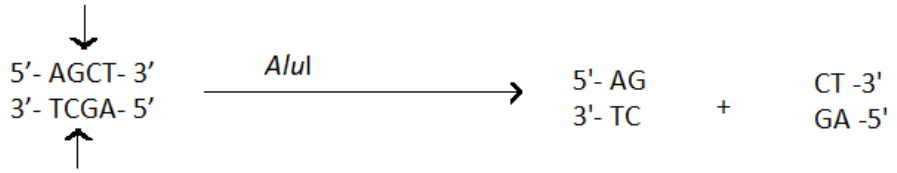
Nomenclature of Restriction Endonuclease

- **First letter – First letter of Genus of the source bacterium** (capitalized).
- **Second and third letter- Species of the bacterium** (italicized).
- **Strain name** (optional, usually italicized).
- **Roman numeral** (I, II, III, etc.) indicating the order of discovery of the enzyme

Example- *EcoR*I- First Restriction Endonuclease discovered in ***E****scherichia* ***co****li* **R**Y13

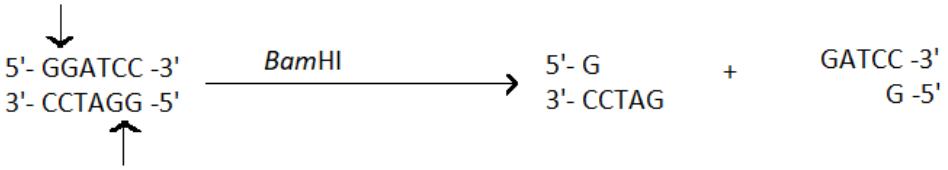
Mechanism of action of Restriction Endonuclease

- Type II restriction endonuclease recognizes the restriction site and then cleaves the phosphodiester bond between the nucleotides within the restriction site.
- These cuts may be symmetrical or asymmetrical.
- Symmetrical cuts or blunt or flush ends do not have any unpaired bases or the bases of the palindrome are paired with their complementary bases in the other strand.



- Asymmetrical cuts or cohesive or staggered or sticky ends have unpaired bases after restriction digestion. They are called cohesive ends as they can base pair easily as the ends are complementary to each other.

Note that some enzymes create 5' overhangs as shown here with *Bam*HI.



Uses of Restriction Endonuclease

a. Gene cloning- digestion of vector and gene of interest.

b. Study of variations in the human genome like polymorphisms.

c. DNA fingerprinting based on RFLP- restriction fragment length polymorphism. Individual genome has unique sequences such that when the genome of two different individuals is subjected to the restriction digestion with the same Restriction Endonuclease, the length of the fragments would vary. When these fragments are run in an agarose gel, the fragments arrange themselves according to their size. This arrangement created a specific pattern on the gel unique to the individual like a fingerprint.

Other enzymes used in RDT

Enzyme	Source	Function
DNA Ligase	Bacteriophage T4	It is used to ligate the DNA fragments. It joins 5'- Phosphate group and 3'- OH group of adjacent DNA fragments by phosphodiester bond.
Alkaline Phosphatase	Bacteria or Calf intestine	Prevents self-ligation of vector DNA by removing 5'- Phosphate group.

Vectors- Carrier DNA molecule

DNA molecules that can be used to carry the insert DNA into the host cell that can replicate in the host cell so that the insert may be cloned or expressed in the host cell. These may be based on plasmid or viruses.

Essential features of vectors are-

a. It must contain the origin of replication (ori) of the host cell so that it can replicate in the host cell.

b. It should contain 2 selectable markers so that the selection of the host cell with the recombinant vector can be done.

c. It must contain a unique restriction site which will allow the insertion of insert DNA. Multiple cloning sites or polylinkers contain multiple restriction sites for many restriction endonucleases. This gives the flexibility in selecting Restriction Endonuclease.

d. It should be of a small size so that it is easy to transfer into the host cell.

Vector	Selectable Markers	Host	Selection method	Insert size	Function	Special features
pBR322	Ampicillin resistance Tetracycline resistance	*E.coli*	Replica plating	< 10 kb	Cloning	
pUC family	Ampicillin resistance Lac Z	*E.coli*	Blue- white selection	< 10 kb	Cloning and Expression	Multiple Cloning Site present
YEp	Ampicillin resistance LEU2	*E.coli* & yeast	Selection based on nutritional requirements	About 10 kb	Shuttle	Contains ori site and selectable markers for both *E.coli* & yeast
λ- phage		*E.coli*		9-23 kb	Viral vector for cloning	Cos site
M-13	Lac Z	*E.coli*	Blue- white selection	2 kb	Viral vector for cloning	Single stranded DNA
Cosmid	Antibiotic resistance	*E.coli*	Based on antibiotic resistance	30-45 kb		Cos sites and plasmid DNA
YAC	TRP1(yeast) Ampicillin resistance (Bacteria) URA3 (yeast) SUP4	Yeast and *E.coli*	Red colonies are selected	250-1000 kb	Cloning, Shuttle	Bacterial origin, ARS, centromere, and telomeres
BAC	Lac Z Antibiotic resistance	*E.coli*	Blue-white selection	50-500 k	Cloning	
Ti plasmid	Antibiotic resistance	*E.coli* and plant cell	Based on antibiotic resistance		Expression	Naturally found in *Agrobacterium tumefaciens*, a soil bacterium and causes crown gall. Transfers its TDAN into the hos plant genome

Host Cells

- Living organisms (cells) used to clone the insert (Fragment of DNA) or express the gene of interest are called host cells.
- Host cells may be prokaryotic or eukaryotic.

Merits of Prokaryotic host cells- *E. coli*

- Genetic make-up is extensively studied.
- Easy to grow and manipulate and can accept a range of vectors.
- It has been extensively studied for safety.
- Doubling time is 20 mins under optimal conditions; makes it possible to clone large amounts of DNA and large amounts of recombinant protein can be produced under appropriate signals.

Merits of Eukaryotic host cells- Yeast

- Simplest of eukaryotic cells (unicellular).
- Extensively characterized genetically.
- Easy to grow and manipulate.

Eukaryotic host cells- Plants and Animals

- These cells can be grown in tissue culture.
- Can be manipulated to form transgenic plants and transgenic animals.

Making rDNA

Selection Methods

Selection methods depend on-

a. Expression of selectable marker genes.
b. Insertional inactivation of selectable marker genes.

Selectable markers	**Method of Selection**
Amp^R gene and Tet^R gene	Replica plating method
Amp^R gene and LacZ gene	Blue- White Selection
LEU2 gene	Dependence or independence on a nutrient- Leucine
GFP gene	Green Fluorescence Protein (Fluorescence under UV Light)

Replica plating Method- pBR322

pBR322 has 2 selectable markers – amp^R and tet^R.

Tet^R gene has a recognition site for *Bam*HI. When the recognition site of a vector is cleaved for incorporating insert DNA this site the tet^R gene gets inactivated. This mechanism is called ***insertional inactivation***.

Selection medium- Nutrient medium (master plate), Nutrient medium + Ampicillin and Nutrient medium + tetracycline

The host cell of *E. coli* subjected to transformation may have any of the following three situations-

a. Host cell with recombinant plasmid- shows only ampicillin resistance and no tetracycline resistance

b. Host cell with plasmid without the insert- shows both ampicillin and tetracycline resistance

c. Host cell without any plasmid- shows resistance to none of the resistance

Blue- White Selection- pUC19

pUC has 2 selectable markers – amp^R and lacZ genes. LacZ gene contains Multiple Cloning Site (MCS). When this vector is cleaved/cut for insert DNA the lacZ gene gets inactivated. This mechanism is called ***insertional inactivation.***

Selection medium- Nutrient medium + Ampicillin + X Gal (substrate for β galactosidase).

The host cells *E. coli* subject to transformation may have any of the following three situations-

a. Host cell with recombinant plasmid- shows only ampicillin resistance and inactive lac Z gene (no beta galactosidase enzyme is produced, so X-gal in the medium is not metabolized) resulting in white-coloured colonies.

b. Host cell with plasmid without the insert- shows ampicillin resistance and active lac Z gene (beta galactosidase enzyme is produced, so X-gal in the medium is metabolized) - resulting in white-coloured colonies.

c. Host cell without any plasmid- no ampicillin resistance and no lac Z gene. Host cells will not grow in the medium.

PCR – Polymerase Chain Reaction

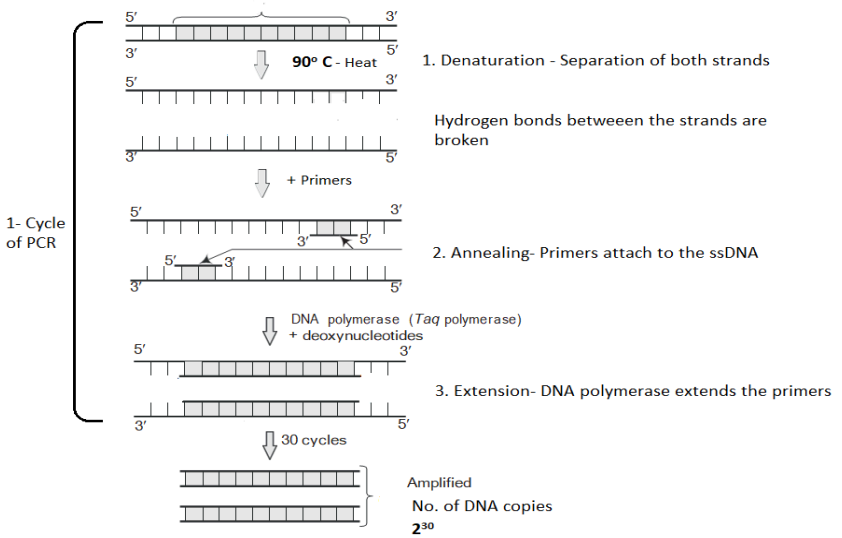


Figure: Polymerase Chain Reaction

Calculation of Amplification of DNA by PCR: No. X 2^n**,** where 'No.' - represents the initial number of DNA molecules.

DNA Sequencing Methods

1. Dideoxynucleotide Chain Termination Method- invented by Fred Sanger and Andrew Coulson

2. Chemical degradation method- invented by Walter Gilbert and Allan Maxam

Dideoxynucleotide Chain Termination Method

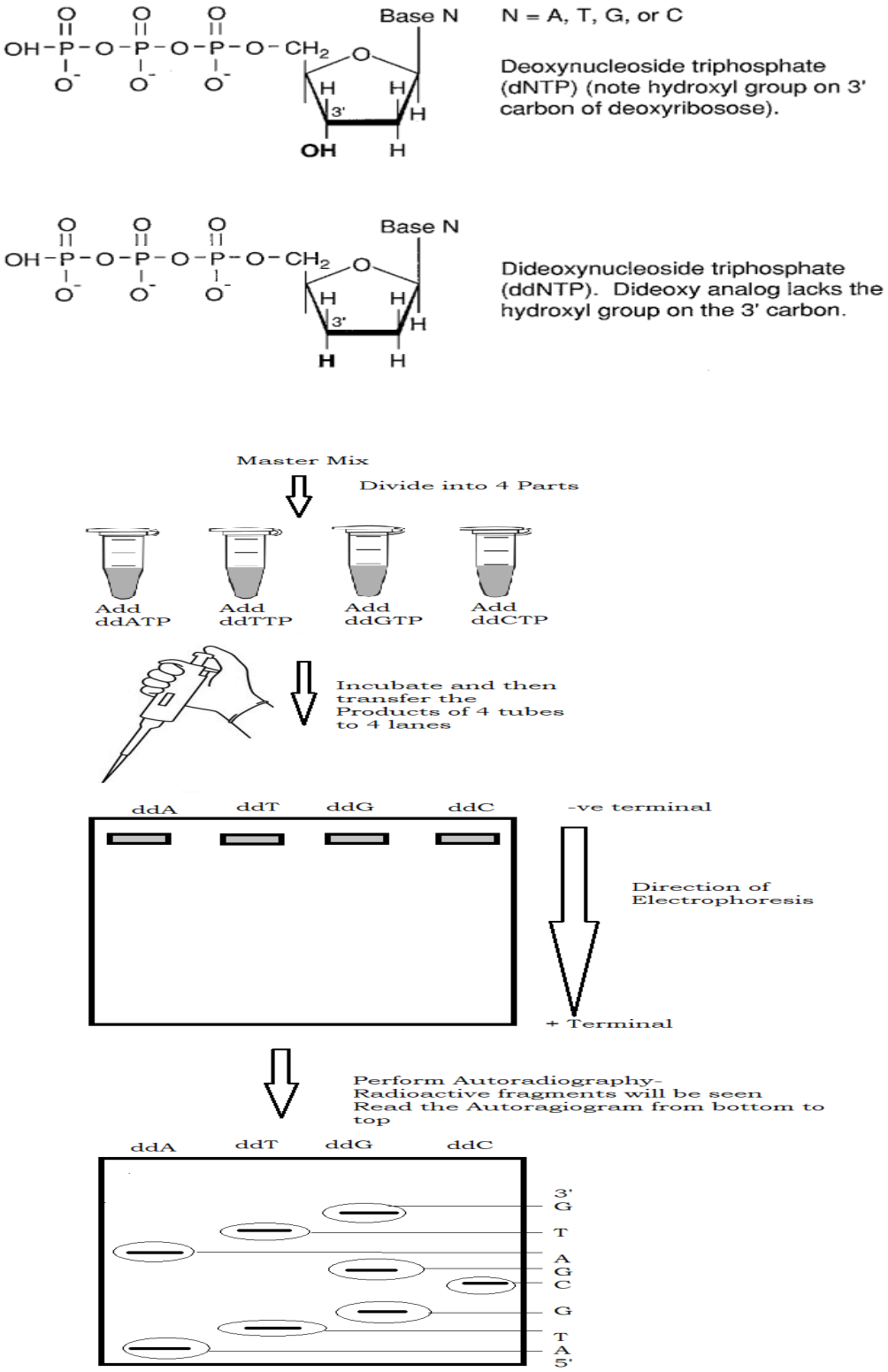


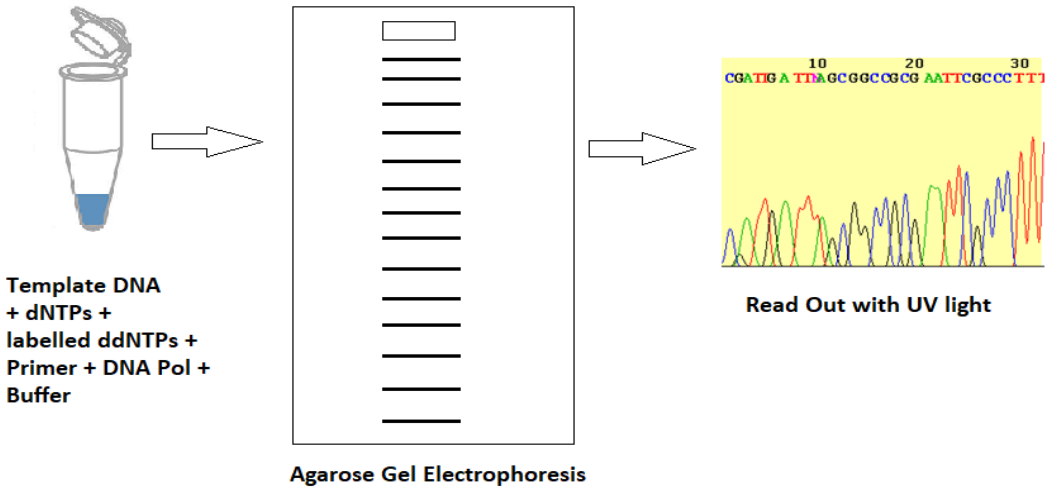
Figure: Sanger Method of DNA Sequencing

Single Tube Sequencing

Principle - based on dideoxynucleotide chain termination method.

Requirements - Template DNA, dNTPs, labelled ddNTPs (with 4 different fluorescent dyes), DNA polymerase, primer and buffer.

Procedure -All the constituents are added to the same tube unlike Sanger method this is why this process is called as **single tube sequencing**. After the process of polymerization, the products are separated according to their size by gel electrophoresis. Then each fragment is read from the bottom (smallest) fragment to the top (largest) with UV light. Fragments show different colour corresponding to the fluorescent tagged ddNTPs. The read out is generated with the sequence of nucleotides according to the colour detected.



Solved Questions-

Multiple Choice Questions

1. Which of the following is the correct pair of scientists and their contribution to RDT?

a) Hebert Beyer, Paul Berg and Stanley Cohen- First recombinant DNA molecule

b) W Arber, H Smith and D Nathans – Discovery of Restriction Endonuclease

c) Mandel and Higa - Preparation of competent cells of Yeast

d) Karry Mullis - Polymerase Chain Reaction

a. I, II and III
c. I, II and IV
b. II, III and IV
d. I, III and IV

2. Which of the following sets of enzymes are used in RDT?

a. Restriction endonuclease, DNA Ligase and Alkaline phosphatase

b. Restriction exonuclease, DNA Ligase and Alkaline phosphatase

c. Restriction endonuclease, RNA Ligase and Alkaline phosphatase

d. Restriction endonuclease, DNA Ligase and DNA polymerase

3. In an RDT experiment, the vector is digested with a restriction endonuclease and then it is treated with alkaline phosphatase (AP). Why is this done?

a. AP is used to remove 3'-OH group to stop self-ligation of vector

b. AP is used to remove 5'-OH group to stop self-ligation of vector

c. AP is used to remove 5'-phosphate group to stop self-ligation of vector

d. AP is used to remove 3'-phosphate group to stop self-ligation of vector

4. You want to prepare an expression vector for a plant cell (host). Which of the following characteristics should be present in the vector?

a. Ori site of the host, selectable marker genes and multiple cloning sites

b. Ori site of the host and promotor and terminator of host

c. Selectable marker genes and ori site of the host cell

d. Multiple cloning site with selectable markers

5. Which of the following vectors cannot be propagated in *E. coli*?

a. pUC19

b. M13

c. pBR322

d. Ti plasmid

6. Why must host cells be treated to become competent?

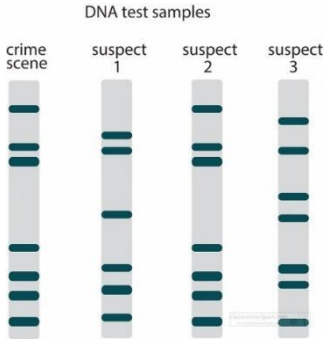
a. Cells do not readily take up DNA from their surroundings.

b. Cells need to be treated with cold calcium chloride solution.

c. Transient pores are created in the membrane due to electric current.

d. *Agrobacterium tumefaciens* is a natural genetic engineer of plants.

7. DNA fingerprinting of the samples found at a crime scene is shown in the figure given here. Which of the suspects is like the DNA fingerprint as that of the sample found at the crime scene?



a. Suspect 1

b. Suspect 2

c. Suspect 3

d. All of these

8. Which of the following DNA fragments will not be digested by a REase?

a. AACCGTAGCTGAT

b. CTGGATCCAATCT

c. CTGGTCGACCTGC

d. ACGGTCGAGCTTT

9. A restriction endonuclease was discovered in *Haemophilus aegyptus*. This is the third enzyme to be discovered. What will be the correct nomenclature of this restriction endonuclease?

a. HaeIII

b. *Hea*III

c. HaeII

d. ***Hae*III**

10. Which of the following are **not** characteristics of selectable marker gene?

a. They confer a special characteristic to the host cell.

b. They can accommodate an insert or foreign DNA.

c. They have a unique RE digestion site or MCS.

d. They have properties that help in DNA replication.

11. Which of the following may be used as a probe for the sequence - ATTGCGATCGATCGTAGCAGT?

a. CGATCGTAGC e.

b. GCTAGCATCG

c. GTAGCAGT

d. ATTGCGATCG

12. Which of the following vectors is can accommodate an insert of more than 1 Mb in size?

a. cosmids

b. Ti plasmid

c. BAC

d. YAC

13. Which of the following enzymes is part of the RM system that protects the host DNA from the action of Restriction Endonuclease?

a. Alkaline phosphatase

b. DNA Ligase and Alkaline phosphatase

c. Methylase

d. DNA polymerase

14. Cosmids are called so because they contain ___ site of ____ and certain features of _____.

a. Cos, lambda phage, and plasmid

b. Cos, M13 phage, and plasmid

c. Cos, restriction endonuclease, and plasmid

d. Cos, bacteriophage, and plasmid

15. Which of the following is considered a natural genetic engineer of plants?

a. Escherichia coli

b. M13 virus

c. Gemini Virus

d. Agrobacterium tumefaciens

16. DNA fingerprinting is used to solve paternity cases. Which of the following is not true about DNA fingerprint of individuals?

a. DNA fingerprint of individuals are unique to the individual.

b. DNA fingerprint of identical twins is same.

c. DNA fingerprint of father and son shows similarities.

d. DNA fingerprint of unrelated individuals shows similar patterns.

17. Which of the following is not an application of PCR?

a. Detection of pathogen

b. Forensic applications

c. Expression of gene

d. Establishing relations from human remains found in mummies.

18. Which of the following is the correct sequence of expression of eukaryotic gene in prokaryotes?

a. Isolation of mRNA reverse transcription (cDNA) insertion of cDNA into cloning vector transformation of host cell selection of transformed cells

b. Isolation of mRNA reverse transcription (cDNA) insertion of cDNA into expression vector transformation of host cell selection of transformed cells

c. Isolation of gene reverse transcription (cDNA) insertion of cDNA into cloning vector transformation of host cell selection of transformed cells

d. Isolation of mRNA insertion of cDNA into cloning vector reverse transcription (cDNA) transformation of host cell selection of transformed cells

Assertion and Reason type Questions

The following questions consist of two statements – Assertion (A) and Reason (R). Answer these questions selecting the appropriate option given below:

a. Both Assertion and Reason are true and the reason is the correct explanation of the assertion

b. Both Assertion and Reason are true but the reason is not the correct explanation of the assertion

c. Assertion is true but Reason is false

d. Both Assertion and Reason are false

19. **Assertion-** A shuttle vector can replicate in both the prokaryotic and eukaryotic host cell.

Reason- YEp can replicate in both *E. coli* and yeast.

Ans- b.

20. **Assertion-** DNA probes are small sequences used to locate a specific fragment of DNA or genes.

Reason- Single stranded DNA binds to other DNA molecules by hydrogen bond on the basis complementary bases.

Ans- a

21. **Assertion-** Viruses are used to clone genes in mammalian cells.

Reason- Viruses have the natural ability to adsorb into host cells and introduce its genes into the host cell for replication.

Ans- a

22. **Assertion-** Vectors are used for transfer of DNA fragments into the host cell.

Reason- Vectors are RNA molecules that can propagate themselves inside the host cells.

Ans- c

23. **Assertion-** cDNA is used to express eukaryotic genes in prokaryotic cells.

Reason- Prokaryotic cells lack the mechanism to remove introns or splicing.

Ans- a

24. **Assertion-** Southern blotting technique is used to identify a specific sequence of RNA.

Reason- Northern blotting technique is used to identify a protein.

Ans- d

25. **Assertion-** PCR can be used to detect pathogens.

Reason- Primers are designed in a manner that the DNA of the pathogen is selectively amplified.

Ans- a

26. **Assertion-** Type I and type III restriction endonucleases are not used for RDT experiments.

Reason- Type II restriction endonucleases are used for RDT experiments.

Ans- b

27. **Assertion-** M13 vector is used to clone DNA fragments for Sanger method of sequencing DNA.

Reason- M13 based vectors can be obtained in the single stranded DNA.

Ans- a

Short Answer type Questions

28. Name any 4 products produced using RDT

- Human insulin in *E.coli*
- Insect resistant cotton- Bt- cotton
- Biodegradable plastics in bacteria or plants
- Hepatitis B vaccine in yeast

29. What is the role of the following enzymes in RDT?

a. Restriction Endonuclease- Digests the DNA (both- insert and vector) so that DNA from two different sources can be joined together.

b. Alkaline phosphatase- Removes the 5'-Phosphate group from the vector to prevent the self- ligation of the vector.

c. Ligase- Joins the DNA fragments forming phosphodiester bonds between 3'-OH and 5' phosphate groups.

30. Mention the essential features of vectors.

- Presence of ori site (origin of replication) of the host cell
- Presence of 2 selectable marker genes
- Presence of unique restriction sites or multiple cloning site (MCS)
- Small size

31. What are the disadvantages of using *E. coli* for production of eukaryotic proteins?

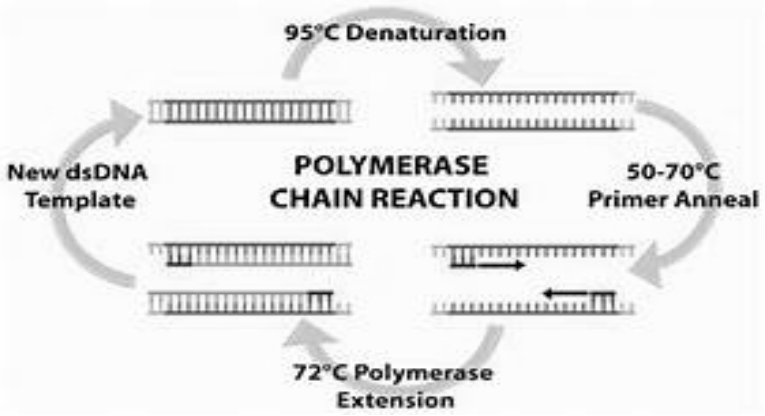
Ans- *E. coli* is a bacterium (prokaryote) and does not have a splicing mechanism and the post translational modifications are not same as that of eukaryotes. Lack of these features are the major disadvantages of *E.coli* to produce eukaryotic proteins.

Long Answer type Questions

32. Discuss the step of PCR with diagram

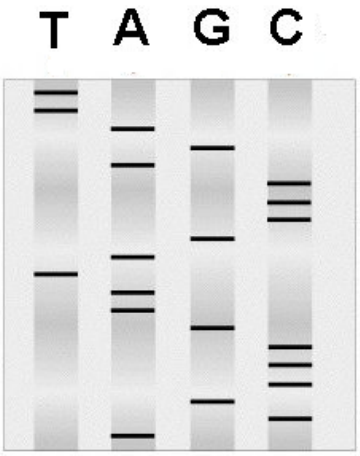
Ans-

Step	Temperature	Purpose
1. Denaturation	~94°C	dsDNA is separated into single strands are separated by breaking hydrogen bonds between the bases.
2. Annealing	~50–65°C	Primers binds (anneal) to complementary template strand on the single-stranded DNA according to the base pairing rules.
3. Extension	~75°C	Taq DNA polymerase synthesizes new DNA by adding dNTPs from primers.



33. The sequence of DNA from an autoradiogram is 5'-ACGCCCGAATAGCCCAGATT-3'. Draw an autoradiogram to show this readout. Write the sequence of template DNA. How many products are formed due to ddTTP and ddATP?

Ans- Diagram of an autoradiogram-



Sequence of template strand- 3'-TGCGGGCTTATCGGGTCTAA-5'

No. of products formed due to ddTTP- 3 and ddATP- 6

Case Based Questions

34. **Study the given table and answer the following questions:**

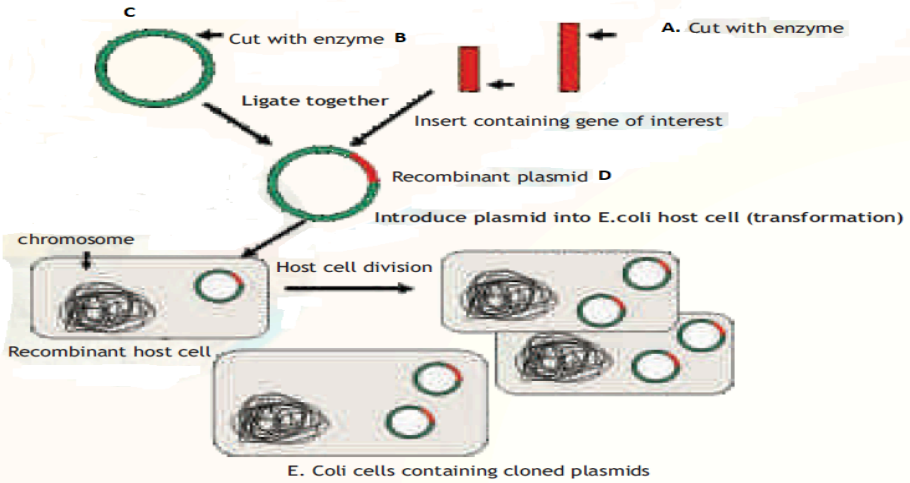


Fig. 1. Schematic representation of the basic steps in RDT.

[1+1+2]

a. If C has selectable makers – Amp^R and LacZ genes and LacZ gene contains MCS. Which selection method will you use to select the recombinant host cells?

Ans- Blue- white selection method

b. Mention the composition of the medium that you will use for selection.

Ans- Nutrient medium + ampicillin + X- Gal

c. State the principle of selection method.

Ans- Expression of Amp^R gene which will confer antibiotic resistance to ampicillin in the medium.

Insertional activation of lacZ gene will turn the colony white in colour.

OR

You digested the plasmid with *EcoR*I and your friend digested the plasmid with *Alu*I. How will this impact the formation of recombinant plasmid?

Ans-

Digestion with *EcoR*	Cohesive ends are formed	Easier to ligate the insert DNA with vector
Digestion with *Alu*I	Blunt ends are formed	Ligation is not easier as no unpaired bases

35. Vector

A popular series of plasmid cloning vectors is the pUC family. These vectors contain a region of the *lacZ* gene that codes for the enzyme β-galactosidase. This region also contains a polylinker and thus insertion of a foreign DNA into any of the restriction sites will result in an altered non-functional enzyme.

a. What does the 'p' in pUC 19 signify? Which property of 'p' is significant in designing these vectors?

Ans- 'p' stands for plasmid which denotes the origin of the vector. Plasmids can self-replicate and maintain high copy number.

b. What is an MCS? Name any two restriction enzymes that will produce staggered ends in the given vector.

Ans- Multiple cloning site contain multiple restriction sites. EcoRI and BamHI will produce staggered ends.

c. Name the components that you will add to the selection medium and why?

Ans-

Components in the selection medium	**Reason**
i. Ampicillin	Expression of AmpR gene will help in selection of transformed host cells.
ii. X-Gal	To detect the expression of lacZ gene

Or

Which host cells can be transformed with this vector? Which selection method will you use to select the transformed cells and why?

Ans- Host cell- *E.coli*.

Selection method- Blue White selection method. This plasmid contains lacZ gene which produces β- galactosidase. The selection medium contains X-gal which when metabolized by this enzyme makes the colony blue and in case of the recombinant plasmid this gene is inactivated and the colony will be white.

UNIT- 5

Protein and Gene Manipulation

Chapter 2: Protein Structure and Engineering

Introduction

All proteins are made up of 20 different amino acids. Proteins differ from each other in their size, sequence and combination of amino acids. These different sequences of amino acids lead to a difference in 3-D structure which in turn leads to variation in functions. Proteins have the most diversity in function among all the classes of biomolecules in our body.

Proteins	Functions
Actin and Myosin	Muscle contraction and relaxation
Haemoglobin	Transport of oxygen
Collagen	Provides strength to bones and tissues

Absence or presence of mutated proteins may lead to change in the function of the protein which may manifest itself as a disease.

Absence of Proteins	Diseases
Lack of α or β subunits of Haemoglobin	Thalassemia
Absence of ADA (Adenosine Deaminase)	Severe Combined Immunodeficiency (SCID) in babies

Mutation of Proteins	Diseases
Mutation in the β subunit of Haemoglobin	Sickle cell anemia
Prion (rogue proteins)	Mad cow disease

The study of proteins with respect to its structure and function becomes essential to understand cellular functions and health of an individual.

Structure Function relationship in Proteins

Various forces like the covalent bonds, hydrogen bonds, van der Waal's forces and hydrophobic bonds keep the 3-D structure of the proteins intact inside our body. A correctly folded protein can function normally. Let's understand this with the help of Chymotrypsin (a proteolytic enzyme) in our body.

Chymotrypsin, a proteolytic enzyme

Basic Stats: It is a linear polypeptide chain made up of 245 amino acid residues, containing 3 peptide chain- A, B and C. Folds to form globular structure.

Type: Serine protease (a class of proteolytic enzymes).

Catalytic triad: Three amino acids come together due to the folding of the protein- His 57, Asp 102 and Ser 195. This results in charge relay system and the serine that has a pKa of 12 becomes acidic.

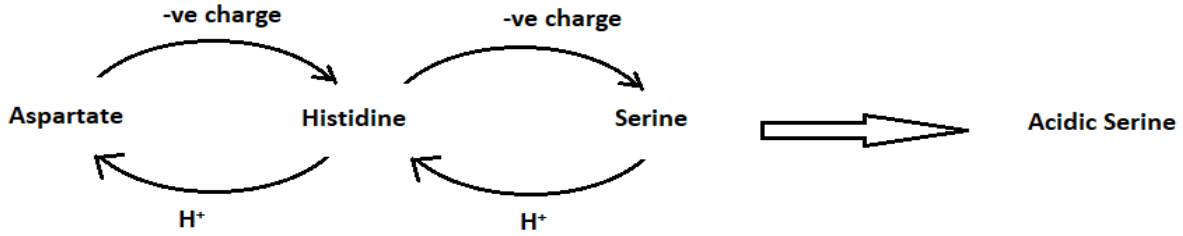
Source: Produced in the pancreas as an inactive precursor or zymogen (**chymotrypsinogen**) and activated in the small intestine.

Activated by: Trypsin in the duodenum (in situ activation).

Function: Breaks peptide bonds in proteins, specifically at the carboxyl side of bulky aromatic amino acids like **tryptophan, phenylalanine**, and **tyrosine**.

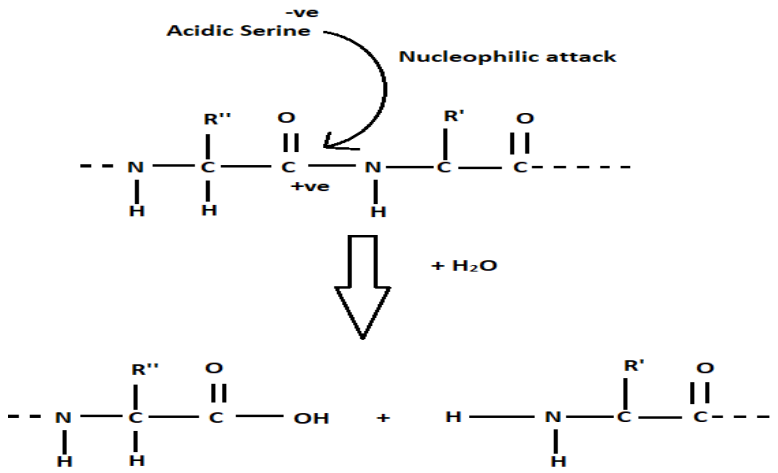
Mechanism: Charge relay system works with the catalytic triad-

1. **Aspartate 102 (Asp102)** – becomes negatively charged due to loss of H^+.
2. **Histidine 57 (His57)** – Asp hydrogen bonds with His, partially borrowing H^+ from His and transferring its negative charge partially.
3. **Serine 195 (Ser195)** – Histidine makes good of the loss of H^+ by attracting H^+ from serine. Thus, serine becomes negatively charged or acidic. This acidic serine acts as the **nucleophile** that directly attacks the carbonyl carbon (C=O) in the peptide bond.



Role of acidic serine in breaking the peptide bond

- In acidic serine residue the negatively charged oxygen anion is able to make a nucleophilic attack on the carbonyl carbon of the peptide bond of its substrate, loosening it so that a water molecule can hydrolyze the bond.



Other Examples of Serine Proteases-

Enzyme	Function	Where It's Found
Trypsin	Cleaves peptide bonds after basic amino acids (Lys, Arg)	Small intestine (from pancreas)
Thrombin	Key enzyme in blood clotting; activates fibrinogen	Blood (produced from prothrombin)
Tissue Plasminogen Activator (tPA)	Converts plasminogen to plasmin	Endothelial cells
Subtilisin	Bacterial serine protease; used in laundry detergents	Bacteria (e.g., *Bacillus subtilis*)

Organophosphates and their action on enzymes with acidic serine.

Nerve gas is a serine alkylating agent which inactivates the brain enzyme acetylcholinesterase by alkylating acidic serine. This may lead to death as well.

Malathion and parathion are used as mosquito repellents that affect the nerve transmission in insects.

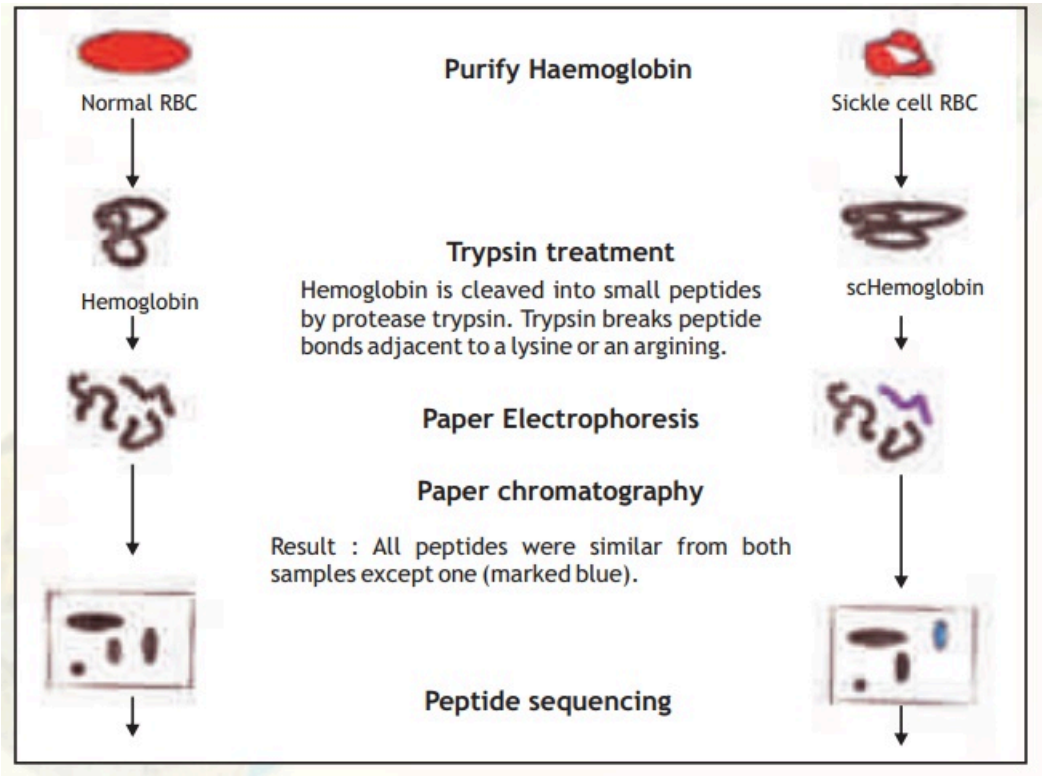
Molecular Disease- Sickle Cell Anaemia

Sickle cell anemia is the first described molecular disease, caused by a single nucleotide mutation.

Linus Pauling studied the electrophoretic mobility of normal and sickle haemoglobin. He observed that the normal Hb moved faster than the sickle cell Hb. This led to the conclusion that both the molecules differed in charge.

This was confirmed by V M Ingram in 1957 at LMB, Cambridge, UK by protein fingerprinting or peptide mapping.

Protein Fingerprinting – Peptide Mapping



Principle - This technique involves the generation and analysis of 2-D patterns produced by separating peptides by two separate techniques like paper electrophoresis and paper chromatography performed at 90° to each other. These techniques create a pattern of peptides which is characteristic to the protein. A difference in a single amino acid will create a different pattern.

Method- The sequence of steps are follows-

a. Both types of haemoglobin are separately collected and digested with trypsin which cleaves the protein after basic amino acids residue Arginine and Lysine.

b. Two strips of Whatman filter paper are spotted with the digested peptides of both samples. Then the peptides are allowed to separate by electrophoresis at pH 2.0. Highly charged peptides will migrate more towards the anode / cathode.

c. Paper strips are dried and chromatography is performed at 90° to the electrophoresis. The solvents used are butanol, water and acetic acid. The peptides will separate based on relative hydrophobicity of the peptides. More hydrophobic peptides will move with the solvent to a longer distance.

d. The chromatograms are dried and stained with suitable reagents like Ninhydrin and the peptides will appear as orange yellow spots.

e. The peptide map for normal and sickle cell haemoglobin are compared.

Observation- There is only one peptide that shows difference.

On further examination of the peptide it was found that the β-chain of scHb has Valine at 6^{th} position instead of Glutamic acid. A single change in amino acid leads to disease, that's why sickle cell anemia is called molecular disease.

Peptide mapping became a useful technique to compare similar proteins from different sources. The huge amount of information generated due to peptide mapping has been stored in databases.

2-D Gel Electrophoresis

Two different techniques are combined in this procedure – Isoelectric focusing (IEF) and SDS-PAGE

Steps of 2-D Gel Electrophoresis

- Sample preparation- Proteins are solubilized in buffers
- Isoelectric focusing (IEF) - Proteins are separated in a pH gradient gel until they reach their isoelectric point (pI), where their net charge is zero.
- SDS- PAGE- Proteins are further separated according to their molecular weight at 90° to the direction of IEF.
- Staining of Gel- gel is stained with stains like Coomassie Blue, silver stain, or fluorescent dyes
- Analysis of the Gel

Advantages of 2-D Gel Electrophoresis

Proteins are separated according to two properties- charge and mass. This provides a better resolution and can be analyzed better.

Characterization of Proteins

Proteins can be characterized by exploiting many physical or chemical properties. Some of the techniques used are listed below-

a. **SDS-PAGE (Sodium Dodecyl Sulphate–Polyacrylamide Gel Electrophoresis)** → Separates proteins by molecular weight.

b. **Protein Fingerprinting** → Separation of peptides based on charge and hydrophobicity.

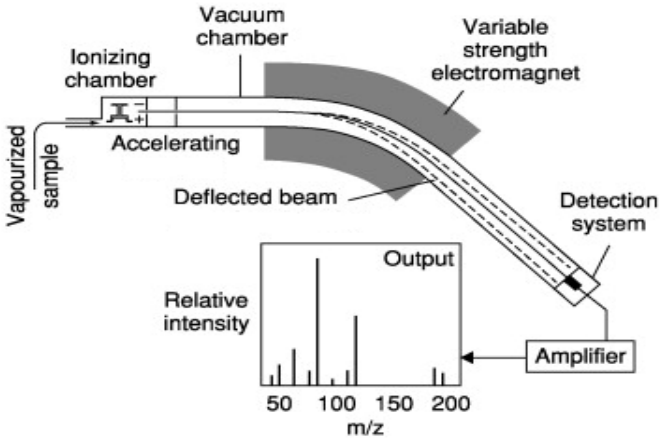
c. **2D Gel Electrophoresis** → Combines IEF and SDS-PAGE for high-resolution protein profiling.

d. **Protein Sequencing** → Edman sequencing to determine the sequence of amino acids

e. **Mass Spectrometry (MS)** → Determines molecular weight and amino acid sequence; detects post-translational modifications.

Mass Spectrometry

Mass spectrometry (MS) is a highly sensitive and precise analytical technique used to measure the mass-to-charge ratio (m/z) of ions. It is widely used in protein identification, characterization, and quantification, and molecular mass of proteins. It can analyse quantities as less as picomoles.



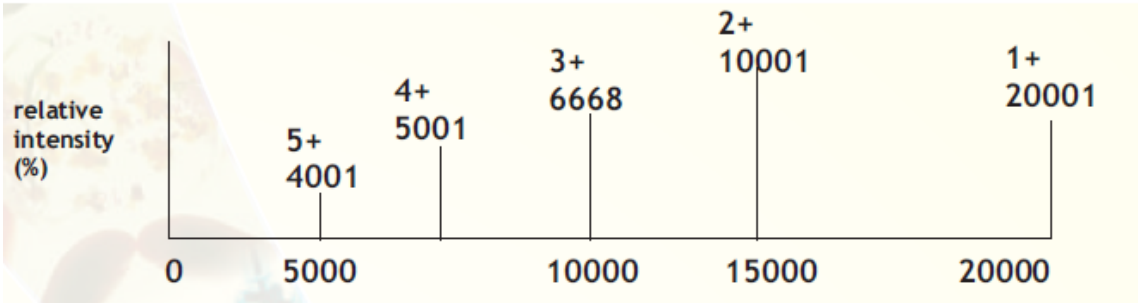
Basic Principle of Mass Spectrometry

- **Ionization** – The sample is converted into charged particles (ions). Ions can be generated by adding protons (H^+). So, mass of the protein increases by 1 and charge on the molecule is +1. This can be done by using MALDI (Matrix Assisted Laser Desorption Ionization). The sample of proteins is introduced in a matrix that is volatilized using a laser. The protein sample gets protonated and vaporized.

- **Mass Analysis** – Ions are separated based on their mass-to-charge ratio (m/z). The ions are introduced into mass analysers in gaseous phase and deflected using electromagnets.

Charge	+1 (H^+)	+2 ($2H^+$)	+3 ($3H^+$)	+n (nH^+)
Mass	M+1	M+2	M+3	M + n
Mass/ Charge	(M+1)/ 1	(M+2)/ 2	(M+3)/ 3	(M+n)/ n

- **Detection** – When the ions reach the detector it records the number and m/z of the ions, generating a mass spectrum based on their abundance.



- X-axis: m/z (mass-to-charge ratio)
- Y-axis: Relative intensity (abundance of each ion)

Each peak represents an ion with a specific m/z. The height (or intensity) of the peak corresponds to how many ions of that m/z were detected.

Example: Simple Peptide Analysis- Calculation of molecular mass

Take the peak at **m/z = 5001 (z = +4)**:

- Multiply by the charge → **5 001**× 4 = 20 004
- Subtract the mass of protons (4 × 1) → 20 004 – 4 = **20 000 Da**

Typical steps to follow for characterization of Proteins-

Crude extract separate in 2-D gel visualize the spots on gel Excise from the gel Mass Spectrometry.

- Separation of proteins can also be achieved by various chromatography techniques.
- Peptides can also be sequenced by the Edman sequencing method.

Protein Based Products

Categories	Proteins	Function
Blood Products and Vaccines	Factor VIII	Treatment of Haemophilia A
	Factor IX	Treatment of Haemophilia B
	Hepatitis B Vaccine	Prevention of Hepatitis
Therapeutic antibodies and enzymes	tPA	Proteolytic enzyme used to digest blocks in myocardial infarction
	OKT-3	Monoclonal antibody used to preven rejection of kidney transplant
Therapeutic hormones and growth factors	Human insulin-produced in bacteria-Humulin	Managing blood glucose level in diabetes
	Platelet derived growt factor	Treatment of skin ulcers in diabetics
Regulatory factors	INF α	Treatment of Hepatitis C
	INF β	Treatment of Multiple Sclerosis
	INF γ	Chronic Granulomatous disease
Analytical Applications	Hexokinase	Quantitative estimation of glucose ir serum
	Uricase	Estimation of uric acid levels in serum
	Horse radish peroxidase and alkaline phosphatase	ELISA (diagnostic technique bases o antibody-antigen interaction)
Industrial enzyme	Alcalase	Soap industry

Categories	Proteins	Function
	Papain	Beverage industry
	Glucose isomerase	Confectionary industry
	Chymosin	Cheese industry
Functional non-catalytic proteins	Whey protein	Emulsification, gelation, water binding, whipping, foaming, etc.
Nutraceutical Proteins	Whey protein concentrates, lactose free milk, infant food formulations	Nutritional requirement of humans, Lactose intolerant babies, food for infants

Designing Proteins (Protein engineering)

Protein engineering is the design and modification of proteins by changing amino acids to improve their catalytic properties or create novel proteins. This can be done by site directed mutagenesis such that any change in amino acids changes the stability of the protein while keeping its function.

The properties that can be changed in proteins are-

a. Improve thermal and pH stability

b. Solvent tolerance

c. Solubility and Catalytic potency

d. Reduce immunogenicity for therapeutic use

Example of Protein engineering- Subtilisin

- Subtilisin is a serine protease secreted by *Bacillus subtilisin*
- Its activity is a result of catalytic triad Asp 32, His 64 and Ser 221. This causes the formation of acidic serine due to the charge relay system.
- Used in laundry detergents as it improves the efficiency of the detergent.
- The native enzyme is inactivated due to oxidation of methionine at 222 position.
- Using site directed mutagenesis, the amino acid at 222 (met) is replaced with other amino acids. The amino acid Ala shows the best result in terms of activity and stability.

Example of Protein engineering- Creation of Novel Proteins

Conventional Vaccines

- Conventional vaccines are inactivated bacteria/viruses or their surface proteins to generate immunity against pathogens.
- Some of these vaccines have undesirable effects like fever or may cause diseases if the inactivation is incomplete.
- These cannot be manipulated like recombinant vaccines.

Recombinant Vaccines

- Specific amino acid sequences in a protein which stimulate the immune system are called epitopes. Recombinant vaccines are designed based on selected epitopes.
- These vaccines have optimal design, scope for micromanipulation.
- These vaccines are safer than conventional vaccines.

Improving nutritional value of cereals and legumes

Essential amino acids are amino acids which are not synthesized by our body and can be procured from food. Cereals and legumes do not have all the amino acids due to deficiencies in the seeds. Supplementing a diet with essential amino acids becomes essential.

Branched chain amino acids (BCAA) are amino acids which have branched side chains. Analysis of whey proteins has shown that it is rich in BCAA. BCAA are essential for biosynthesis of muscle proteins. They help in increasing bioavailability of high complex carbohydrates intake and are absorbed by muscle cells for muscle building.

Theories of BCAA's action- during exercise the BCAA are released from muscles, the carbon skeleton is used as fuel and the nitrogen part is used to make alanine which is converted into glucose in the liver.

So, athletes take BCAA before and after exercise to protect their muscle mass and delay exhaustion.

Biological Value- Biological Value is the percentage of absorbed nitrogen from a protein source that is retained in the body for growth and maintenance.

BV= (Nitrogen retained/ Nitrogen absorbed) ×100

The BV of Whey proteins is the highest compared to rice, wheat, soya and egg proteins.

Protein Efficiency Ratio (PER)- it is used to measure the growth expressed in terms of weight gain of an adult by consuming 1 g of food protein.

PER = Weight gain of test subject (g) / Protein consumed (g)

PER value in decreasing order- whey, milk, casein, soya, rice, wheat.

Modern day approach of overcoming nutritional deficiencies in seeds-

- Engineer genes that encode storage proteins with more essential amino acids by either inserting additional amino acids or substituting existing amino acids with new amino acids.
- Zein storage protein of Maize is being used to enrich maize.
- Introduction of novel proteins rich in essential amino acids is also being considered.

Solved Questions

Multiple Choice Questions

1. Which of the following molecules has the most diverse functions in a cell?

a. DNA
b. RNA
c. Protein
d. Lipids

2. Which of the following will not deactivate a serine protease?

a. Acetylcholine esterase
b. Malathion
c. Parathion
d. Nerve gas

3. Which of the following amino acids is not a branched chain amino acid?

a. Leucine
b. Isoleucine

c. Methionine

d. Valine

4. Whey has been used to treat various ailments since 6000 BC. Why?

a. Whey increases tripeptide glutathione in the cells which protects cells from free radicals

b. Whey decreases tripeptide glutathione in the cells which protects cells from free radicals

c. Whey increases amino acids in the cells which protects cells from free radicals

d. Whey protein can be used as probiotic and a good source of beneficial bacteria

5. Which of the following is not a correct pair of protein and its use?

a. Factor VIII- Haemophilia A

b. Factor VIII- Haemophilia B

c. OKT-3 – Prevention of graft rejection

d. Horseradish peroxidase- ELISA

6. On finding that Hb moves faster than scHb, Linus Pauling predicted that there is a difference of charged amino acid between both. How has this statement proved true?

a. Acidic amino acid (Glutamate) is replaced by non-polar amino acid (Valine)

b. Acidic amino acid (Valine) is replaced by non-polar amino acid (Glutamate)

c. Non-polar amino acid (Glutamate) is replaced by acidic amino acid (Valine)

d. Non-polar amino acid (Valine) is replaced by acidic amino acid (Glutamic acid)

7. Which of the following is not true about protein fingerprinting?

a. Purified proteins are cleaved with trypsin to create fragments.

b. The first technique to separate the peptides at pH 2 is paper electrophoresis.

c. The first technique to separate the peptides at pH 2 is paper chromatography.

d. The second technique to separate the peptides is paper chromatography based on partition coefficient.

8. How is native subtilisin inactivated by bleach?

a. Due to oxidation of amino acids alanine at position 222

b. Due to oxidation of amino acid methionine at position 222

c. Due to oxidation of amino acid serine at position 222

d. Due to oxidation of amino acid phenylalanine at position 222

9. Sickle cell anemia is considered molecular disease-

I. It is prevalent in parts of Asia and Africa where malaria is prevalent

II. It causes the sickling of RBC which impairs its oxygen carrying capacity

III. Change in the shape of hemoglobin is caused due to change in an amino acid in the beta chain of hemoglobin

IV. The beta chain has glutamic acid replaced by valine at its 6th position thus changing the structure of protein

a. I and II b. II and III c. **III and IV** d. IV and I

10. Which of the following diseases is caused by absence of proteins?

a. Thalassemia

b. Sickle cell anemia

c. Mad cow disease

d. All of these

11. Which of the following protein products is not used for therapeutics-

a. Factor IX

b. Alkaline phosphatase

c. OKT3 and tPA

d. Humulin

12. Which of the following protein products is not used in industries-

a. Chymosin

b. Alkalase

c. Papain

d. Humulin

13. Charge relay system functions due to –

a. The catalytic triad Glu-His- Ser

b. The catalytic triad Asp-Tyr- Ser

c. The catalytic triad Asp-His- Ser

d. The catalytic triad Asp-His- Val

14. The chromatograms of peptide mapping are dried and stained with _____ to produce orange yellow spots.

a. Silver stain

b. Ninhydrin

c. Coomassie blue

d. Eosin

15. The correct sequence of steps in peptide mapping is-

a. Purification of protein trypsin treatment paper electrophoresis paper chromatography peptide sequencing

b. Purification of protein trypsin treatment paper chromatography paper electrophoresis peptide sequencing

c. Purification of protein peptide sequencing trypsin treatment paper chromatography paper electrophoresis

d. Peptide sequencing purification of protein trypsin treatment paper chromatography paper electrophoresis

Following question consists of two statements – Assertion (A) and Reason (R). Answer these questions selecting the appropriate option given below:

A. Both Assertion (A) and Reason (R) are the truth and Reason (R) is a correct explanation of Assertion (A).

B. Both Assertion (A) and Reason (R) are the truth but Reason (R) is not a correct explanation of Assertion (A).

C. Assertion (A) is true and Reason (R) is false.

D. Assertion (A) is false and Reason (R) is true.

16. **Assertion**- Sickle cell anaemia is a disease prevalent in parts of Africa and India where malaria is also endemic.

Reason- Sickled RBCs resist malarial infection and hence offer some selection for malaria to be co-prevalent with sickle cell anaemia.

Ans- A

17. **Assertion**- Factors VIII and IX are used to treat haemophilia A and B

Reason- Factors VIII and IX are derived from blood.

Ans- B

18. **Assertion** – Conventional vaccines are not safe when compared to recombinant vaccines.

Reason – Conventional vaccines may have proteins that are manipulated to contain epitopes only.

Ans- C

19. **Assertion**- Protein efficiency ratio can be used to measure the growth in terms of weight gain after consuming 1 gram of protein

Reason- Biological value and PER of wheat is the highest when compared to other proteins.

Ans- C

20. **Assertion**- Curd is used as a probiotic which is administered with an antibiotic.

Reason- Curd is a good source of beneficial bacteria that can colonise the intestinal tract after the use of antibiotics.

Ans- A

21. **Assertion**- Humulin is better than pig insulin.

Reason- Humulin takes about 15 mins and pig insulin takes about 3 hours to act.
Ans- A

22. **Assertion** – Cow and buffalo milk can be given to human babies.
Reason- The composition of milk from cow and buffalo are different from each other.
Ans- D

23. **Assertion**- Whey protein causes browning of baked food by Mailard reaction
Reason- Whey protein also tarps water to keep food moist and soft
Ans- B

24. **Assertion**- Prions are misfolded proteins that can cause diseases
Reason – Mutation in proteins in caused due to change in the amino acid sequence
Ans- B

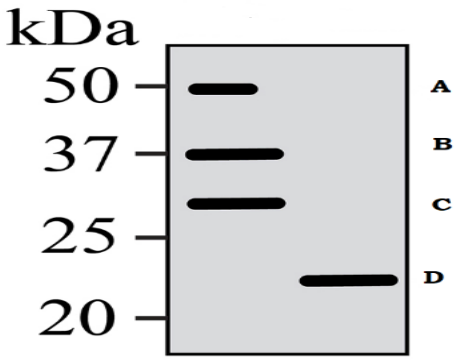
25. **Assertion**- MALDI is used to ionize proteins for mass spectrometry.
Reason- Mass spectrometry is an analytical technique used to characterize proteins.
Ans- B

Short answer type questions

26. What is the consequence if a protein is incorrectly folded? Given an example to illustrate your answer.

If a protein folds incorrectly, it will lead to the formation of prions and lead to disease like mad cow disease.

27. After carrying out SDS PAGE of a protein sample from a cell, the following was observed.



a. Which protein has migrated the most in the gel and why?

Ans- D has migrated the most as it has the smallest molecular weight

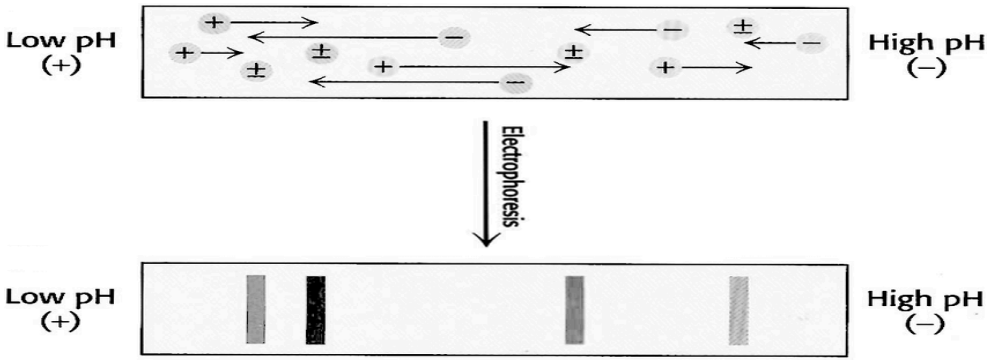
b. Which protein has migrated the slowest and why?

Ans- A has migrated the least as it has the largest molecular weight

28. Nerve gas affects the brain enzyme which is fatal. Which enzyme is affected by this gas and why?

Acetylcholine esterase. This enzyme has acidic serine residue which can be knocked off by serine alkylating agent – nerve gas. Since this enzyme has an important function in the brain and when these enzymes do not function and may become fatal.

29. Observe the figure given below and answer the following questions-



Identify the technique shown in the figure.

Ans- Isoelectric Focusing

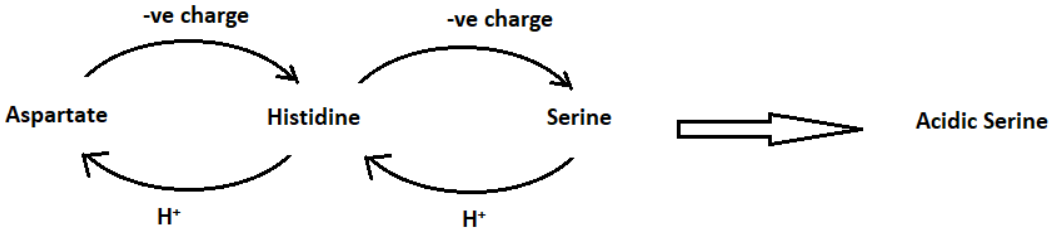
Why do the proteins stop moving in the electric field as shown in the second figure?

Ans- Proteins move to the pH where their net charge becomes zero and the proteins do not move under electric field.

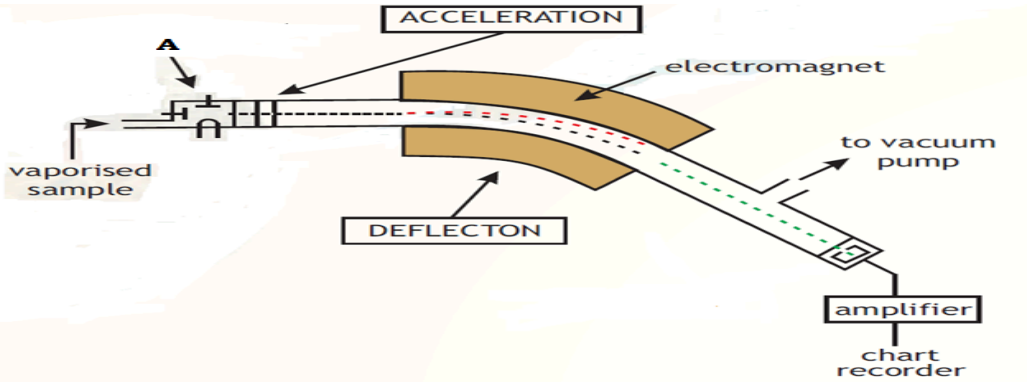
30. What is the role of acidic serine in chymotrypsin? How does the serine residue become acidic despite its pKa is 12.

Ans- Acidic serine being negatively charged makes a nucleophilic attack on the carbonyl carbon of the peptide bond and hydrolyses the peptide bond.

Serine becomes acidic due to the charge relay system.



31. Observe the figure given below and answer the following questions-



Identify A shown in the figure.

Ans- Mass Spectrometry

Mention the method used to achieve A in mass spectrometry.

Ans- A- Ionization method- MALDI is used

32. Name any two enzymes that have charge relay system and their function

Ans- Subtilisin- used in detergent industry

Thrombin- blood clotting factor in humans

33. Novel proteins have been created for various purposes. Give examples of such protein with their use.

Ans- Proteins are the main molecules that act as an antigen. There are specific sequences of amino acids that are known as epitopes. Novel proteins can be designed by using only these epitopes so that safe vaccines can be designed. Example- recombinant vaccines like Hepatitis B vaccine.

34. Why do we need to design and engineer proteins?

Ans- Native or wild type proteins which have some desirable function may get inactivated due to some physical or chemical conditions like temperature difference, pH difference. To stabilize these proteins, we must know the cause of inactivation. Inactivation may be due to a specific amino acid, which may be replaced to increase its stability and retain its function.

Long answer type questions

35. **What is protein fingerprinting? How is this technique useful for the comparative study of proteins? Illustrate with an example and diagrams.**

Ans-Protein fingerprinting is a technique used to identify and characterize proteins by separating peptides and generating a distinct pattern of peptide fragments, much like a "fingerprint" unique to each protein.

This technique can be used to differentiate two proteins by following the steps-

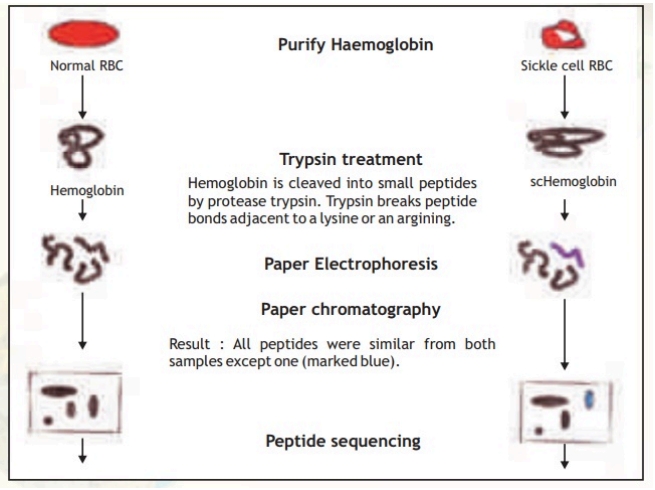
a) Both types of haemoglobin are separated and digested with trypsin which cleaves the protein after basic amino acids Arg and Lys.

b) Two strips of Whatman filter paper are spotted with the digested peptides of both samples. Then the peptides are allowed to separate by electrophoresis at pH 2.0. Highly charged peptides will migrate more towards the anode / cathode.

c) Paper strips are dried and chromatography is performed at 90^{o} to the electrophoresis. The solvents used are butanol, water and acetic acid. The peptides will separate based on relative hydrophobicity of the peptides. More hydrophobic peptides will move with the solvent to a longer distance.

d) The chromatograms are dried and stained with suitable reagents like Ninhydrin and the peptides will appear as orange yellow spots.

e) The peptide map for normal and sickle cell haemoglobin are compared.



36. What is mass spectrometry? Mention the advantages of using this technique to study proteins.

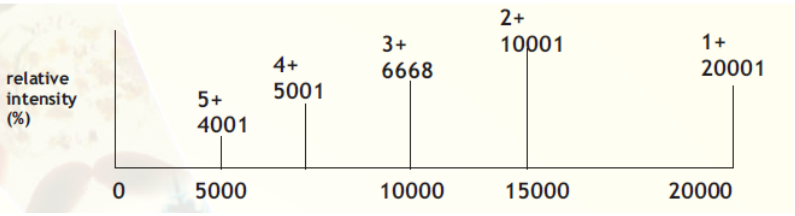


Image shows the read out of a protein with different m/z ratio. What is the molecular weight of the protein? How is the total mass of a charged protein calculated?

Mass spectrometry (MS) is a highly sensitive and precise analytical technique used to measure the mass-to-charge ratio (m/z) of ions. It is widely used in protein identification, characterization, and quantification, and molecular mass of proteins.

Advantage- It can analyse quantities as less as picomoles conc. of proteins.

Molecular wt is 20000 Da. Mass can be calculated by the formula $m/z = M+nH^+/ n$

When z is +1 then the m/z is 20001, M= 20001-1 = 20000 Da

37. What is BCAA? Name any one source that is rich in BCAA. Why are BCAA important for sports persons?

Branched-Chain Amino Acids (BCAAs) are a group of three essential amino acids with a branched molecular structure- leucine, isoleucine, and valine. These amino acids are essential for muscle protein biosynthesis. They also help in increasing the bioavailability of high complex carbohydrates intake and are absorbed by muscle cells. BCAA also reduces muscle breakdown and acts as an energy source before and after exercise.

38. Give an example of a protein that has been engineered for industrial use. Explain briefly how this protein has been engineered.

Subtilisin. It is a serine protease (charge relay system Asp, His and Ser). Used in Laundry detergents to digest stains. It gets inactivated in presence of bleach due to oxidation of Met 222.

To improve the stability, Met222 is replaced with Ala. This makes the enzyme stable and can act as a stain cutter.

This is carried out by the process of site-directed mutagenesis of subtilisin gene in *E. coli*.

Case based Questions

39. Read the following passage and answer the following questions-

The following table shows the constituents of different sources of milk- buffalo, human and cow.

Constituents (per 100 ml of milk)	buffalo	human	cow
1. Protein (g)	3.8	1.2	3.3
2. Casein (g)	3.0	1.4	2.8
3. Lactalbumin (g)	0.4	0.3	0.4
4. Lactoglobulin (g)	0.2	0.2	0.2
5. Fat (g)	7.5	3.8	3.7
6. Lactose (g)	4.4	7.0	4.8
7. Calorific value (K Cal)	100.0	71.0	69.0
8. Calcium (mg)	203.0	33.0	125.0
9. Phosphorous (mg)	130.0	15.0	96.0
10. Chloride (mg)	112.0	43.0	103.0

a. Name two components of all three types of milk which are in similar quantities.

Ans- Lactalbumin and lactoglobulin

b. Why buffalo or cow milk cannot be used instead of human milk to feed human infants?

Ans- The amount of protein, casein is higher in both cow and buffalo which may be difficult for infants to digest.

c. Why are special milk formulations prepared for human infants? Why are these formulations called nutraceutical proteins?

Ans- Special milk formulations are needed for babies who are intolerant to lactose. These formulations are called nutraceutical proteins as they have nutritional and pharmaceutical value.

Or

Why is whey protein considered as nutraceutical protein?

Ans- Whey proteins are considered as nutraceutical proteins as it provides nutrition and has pharmaceutical value. It elevates the levels of tripeptide glutathione in the cell which acts on free radicals and xenobiotics.

40. The following table shows the result of site directed mutagenesis at codon 222 in subtilisin. Study the table and answer the following questions-

Codon-222	% activity w.r.t wild type	Codon-222	% activity w.r.t. wild type
Cys	138.0	Gln	7.2
Met	100.0	Phe	4.9
Ala	53.0	Trp	4.8
Ser	35.0	Asp	4.1
Gly	30.0	Tyr	4.0
Thr	28.0	His	4.0
Asn	15.0	Glu	3.6
Pro	13.0	Lie	2.2
Leu	12.0	Arg	0.5
Val	9.3	Lys	0.3

a. Why is site directed mutagenesis done for the protein subtilisin?

Ans- Subtilisin is used in laundry detergent, and gets deactivated in presence of bleach. It is essential in removal of stains.

b. Why is the codon 222 selected? Which amino acid is present at the position 222 in the wild type subtilisin?

Ans- It gets inactivated in presence of bleach due to oxidation of Met 222.

c. Why are laundry detergents supplemented with enzymes?

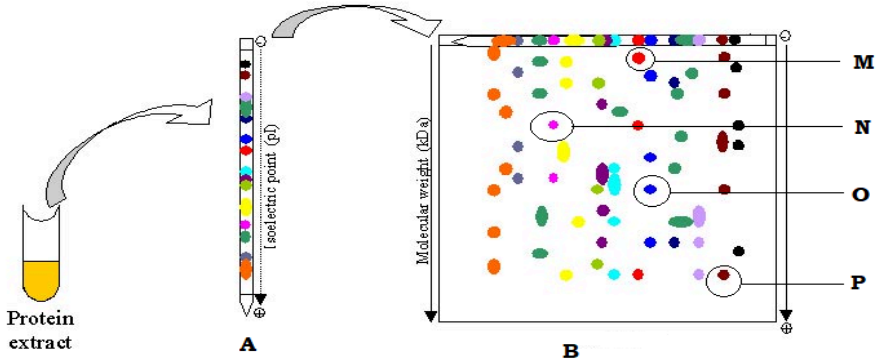
Ans- Laundry detergents are supplemented with enzymes as they are useful in digested stains and makes the process of cleaning quicker and easier.

Or

Mention the mechanism of action of the enzyme subtilisin.

Ans- It is a serine protease. It functions on a charge relay system in the catalytic triad Asp, His and Ser. The negative charge is transferred from Asp to Ser through His. This makes serine acidic and this acidic serine digests peptide bonds.

41. Observe the following diagram and answer the following questions-



a. Why is this technique called 2-D gel electrophoresis?

Ans- It separates the proteins according to their pI and mass at 90° to each other.

b. Proteins M, N, O and P have been separated in B. Arrange these proteins in increasing order of molecular weight.

Ans- P<O<N<M

c. Identify A and state the principle involved in A.

Ans- IEF- Under an applied electric field, proteins migrate through the gel with pH gradient (ampholytes) and move to the position in the pH gradient where its net charge is zero (i.e., its pI).

- Below pI → protein is positively charged → migrates toward the cathode.
- Above pI → protein is negatively charged → migrates toward the anode.
- At pI → no net charge → no movement → "focuses" into sharp bands.

Or

Identify B and state the principle involved in B.

Ans- SDS-PAGE- Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis. This technique is used to separate proteins based on their molecular weight. The protein with least molecular weight moves the fastest and farthest towards anode.

UNIT- 5

Protein and Gene Manipulation

Chapter 3: Genomics, Proteomics and Bioinformatics

Gene Prediction and Counting

Gene prediction by computational methods for finding the location of protein coding regions is one of the essential issues in bioinformatics. It has vast application in structural genomics ,functional genomics , metabolomics, transcriptomics, proteomics, genome studies and other genetic related studies including genetics disorders detection, treatment and prevention.

Gene prediction can be done by creating an algorithm with our existing knowledge of nucleotide structure of genes like promoters, ORFs, start codons, exons, introns, stop codons.

However, there are limitations in these algorithms due to overlapping genes and splice variants which makes it difficult to define the parts of the DNA that should be regarded as the same or several different genes.

Allowing for some experimental errors the number of genes are predicted by computational method and the results are as shown in the table below-

Table 1. Genome size and gene predictions between several organisms.

Organism	No. of chromo somes	Genome size in base pairs	The Number of Predicted genes	Part of the genome that encodes for protein
Bacteria *Escherichia coli*	1	500,000	5000	90%
Yeast *Saccharomyces cerevisiae*	16	12,068,000	6340	70%
Worm *Caenorhabditis elegans*	6	100,000,000	19,000	27%
Fly *Drosophila melanogaster*	4	175,000,000 - 196,000,000	13,600	20%
Weed *Arabidopsis thaliana*	5	157,000,000	25,498	20%
Human *Homo sapiens*	23	3,000,000,000	20,000 - 25, 000	< 5%

Observation from the table-

- Number of genes in humans is surprisingly low when compared to the other organisms in terms of complexity. This reflects unreliability of in-silico methods.
- There is no simple correlation between the complexity of the organism and the number of genes.

Genome Similarity, SNPs and Comparative Genomics

99.8% of the genome of all humans are identical. The difference of 0.2% in the genome is enough to make each individual unique.

Single Nucleotide Polymorphism (SNP) is a variation at a single base position in the DNA sequence among individuals in a population. The variation must occur in at least 1% of the population for it to be considered as SNP.

SNPs occur in-

Region	Effect
Coding region	May change codon and change in amino acids or no change in amino acids or introduce a stop codon.
Non-coding region	Can affect gene regulation, splicing, or have no functional impact.
Intergenic regions	Often neutral but can affect regulatory elements nearby.

There are 1.6 million to 3.2 million sites of SNPs in the human genome.

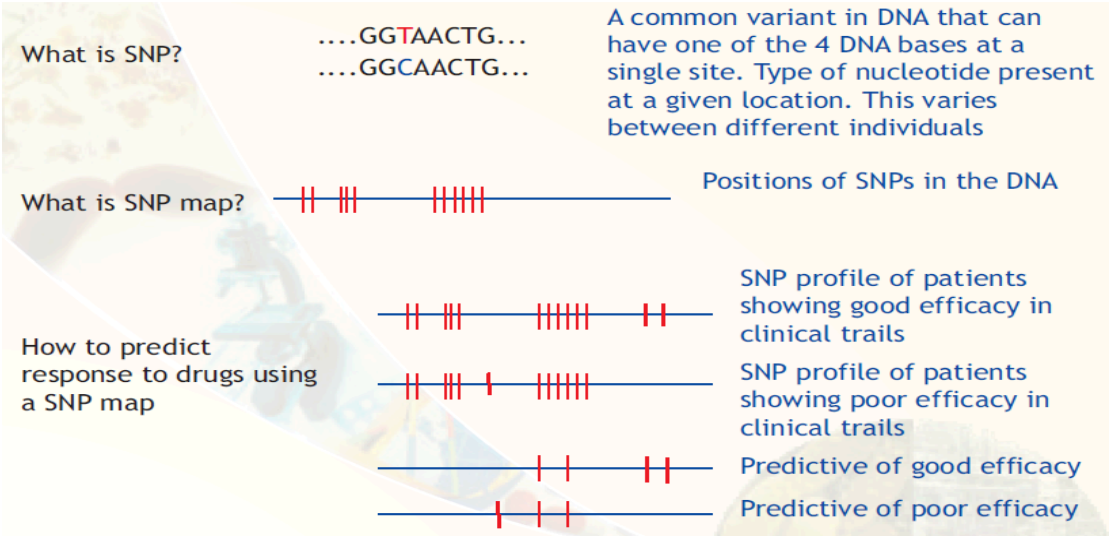
Applications of these variations-

- Variations in the non-coding parts of the genome are exploited in DNA fingerprinting- forensic science.
- Harmful variations- leads to susceptibility to diseases. These variations also determine the severity of the disease or the effectiveness of drugs/ treatments. Single base difference in the ApoE gene is associated with Alzheimer's disease.

Table 2. Genes and diseases

Single-gene mutations which follow mendelian inheritance	Gene polymorphisms which has complex inheritance
Cystic Fibrosis (Cystic Fibrosis Transmembrane Conductance Regulator CFTR gene) 1. Inheritance: autosomal recessive disease	Common late-onset Alzheimer's disease 1. Inheritance: Major cause is epsilon4 allele of the gene coding for apolipoproteinE (APOE)
2. Genomic location: Chromosome 7 (7q31.2)	2. Genomic location: Chromosome 19 (19q13) and recently Chromosome 10 (10q21).
3. Mutation: The most common mutation is a deletion of 3 bps resulting in the loss of codon no. 508, which codes for phenylalanine	
Huntington disease (Huntingtin gene HTT) 1. Inheritance: autosomal dominant 2. Location: Chrosome 4 (4p16.3) 3. Mutation: increased number of CAG repeats more than 35 times	Migraine 1. Susceptibility locus: Chromosome 6p12.2 - 6p21.1 and Chromosome 1q31

- Beneficial variations- simple variations in chemokine- receptor gene CCR5 leads to resistance to HIV infection and development of AIDS.
- SNP maps can be used to determine the effectiveness of drugs by physicians as shown below. The vertical bars represent the location of SNPs. Patients who have a positive response can be identified by their SNP map and patients with negative response are shown in different SNP map.



- SNP analysis can be used to study population genetics.
- Genome differences between related species- Humans share approximately 98–99% of their DNA with chimpanzees, indicating close evolutionary relation. Humans and mice share about 97.5% of functional DNA, reflecting slow genome evolution.

Functional Genomics

Functional genomics is used to-

- Determine function of genes in a cell and their interactions
- Understand gene expression patterns in a tissue specific manner (healthy or diseased)
- Explore gene–protein interactions (how environmental conditions change gene expression with signal transduction and affect cellular processes like cell division)
- Study effects of genetic variation (e.g., SNPs, mutations) for studying diseases, population differences and evolution of species

Some of the techniques used in functional genomics are- FISH, Microarray technology and it is integrated with proteomics as understanding protein is essential in understanding genes and their functions.

FISH- Fluorescence in situ Hybridization

Colour (fluorescent dyes) can be introduced into DNA by a technique called Nick Translation (Rigby & Paul Berg, 1977)

- **DNase I** creates random **nicks** (breaks) in double-stranded DNA.
- **DNA Polymerase I** adds nucleotides to the 3' ends at the nicks, replacing old DNA.
- One of the dNTPs is labeled with a **fluorescent dye** (e.g., red or green).
- The result is **fluorescently labeled DNA**.

Size of DNA fragment with fluorescence after nick translation depends upon the amount of enzyme and incubation time of reaction. The size range can vary from 300 to 3000 bps.

Application of FISH in CML (Chronic Myeloid Leukemia)

Genetic Cause:

- **CML involves a reciprocal translocation between chromosomes 9 and 22 which creates the Philadelphia chromosome (Ph1).**

- **The translocation fuses the bcr gene (chromosome 22) and the abl gene (chromosome 9).**

FISH in CML Diagnosis:

- This can be detected by using FISH to paint chromosome 9 red and chromosome 22 green.
- In CML cells, red and green signals **overlap**, producing **yellow** (indicating fusion).
- **Normal cells** show **separate red and green** spots.
- **It can also be used to monitor treatment effectiveness** (fewer yellow cells = positive drug response).

Microarray Technology

What is Microarray?

- A **DNA chip** containing thousands of DNA spots fixed on a solid surface (glass/silicon).
- Used to study **gene expression** across the whole genome at once

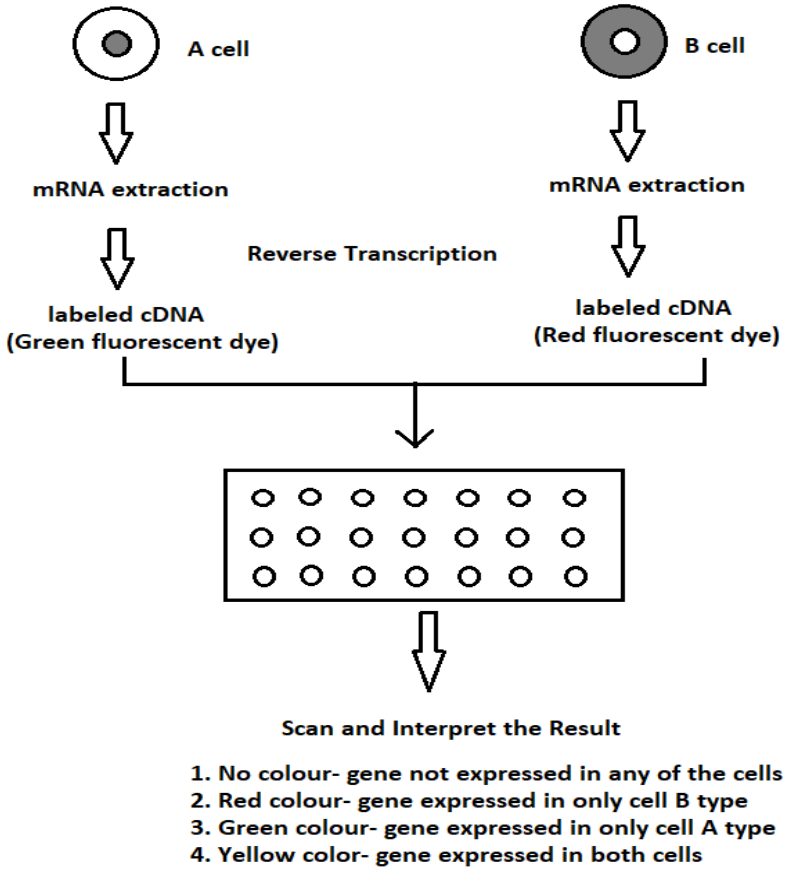
Principle

- DNA sequences are fixed in spots on a solid surface (microarray).
- Single-stranded fluorescently labelled cDNA from samples is added.
- cDNA binds only to complementary (matching) DNA spots.
- Fluorescence intensity and color at each spot show gene activity levels.

Procedure-

Step	**Description**
1. Sample Collection	Extract RNA from two samples (e.g., Cell A and Cell B or healthy vs. cancer tissue).
2. Reverse transcription and Labelling	Convert RNA to cDNA probes by reverse transcription and label each sample with fluorescent dyes (e.g., red for cell A, green for cell B).
3. Hybridization	Apply both labelled cDNAs to the microarray; they bind to complementary sequences present on the microarray.
4. Washing	Remove unbound cDNA.
5. Scanning & Analysis	Use a laser scanner to detect fluorescent signals; compare gene expression levels.
6. Interpretation	• Each spot represents a gene.

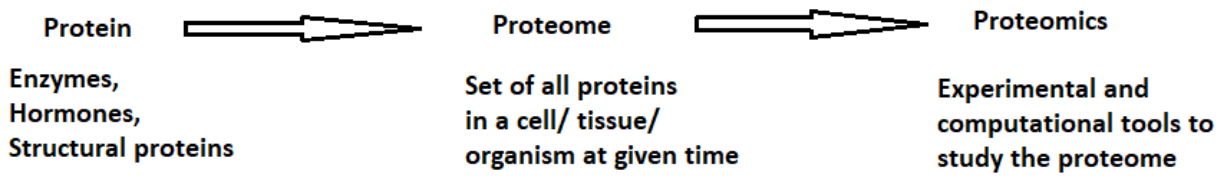
Step	Description
	• Green signal: gene is overexpressed in cell A. • Red signal: gene is overexpressed in cell B. • Yellow signal: equal expression in both samples. • No colour: gene not expressed by any of the cells.



Applications of Microarray

Field	Purpose
Tissue specific gene	Identify genes that are up/down-regulated under certain conditions in different tissues.
Cell cycle variations	Compare the gene expression profile of cells through the progression of cell cycle.
Cellular responses to environment	Study effects of change in physical changes and chemicals or drugs on gene expression.
Genetic disease due to defects in genes	Detect mutations or gene deletions.

Proteomics: Proteome- Complete set of proteins in a cell/ tissue/ organisms. Proteomics refers to large scale characterization of the proteome.



Type of Proteomics	Function
Structural Proteomics	Protein 3D structures and complexes present in a cell or organelle. Identification of all the proteins in a complex and characterize all protein-protein interactions between these proteins.
Expression Proteomics	Protein abundance and expression levels under different conditions or samples. Proteome and sub-proteome can be compared. This can be useful in identification of disease specific proteins like tumour and normal cells.
Functional Proteomics	Protein activity in molecular networks in a cell like nuclear pore complex, interactions, and role in cellular processes.

Genes and Proteins

Relationship between the number of genes and the number of proteins is not linear. **One gene ≠ One protein**

One gene= many different polypeptides due to alternate splicing which results from joining different combinations of exons. Number of genes in humans 20,000 to 25,000 vs Number of proteins 100,000

Reasons for Gene-Protein Mismatch

a. **Post transcriptional modifications**- Alternate splicing (Different combinations of exons are joined to form different mRNA transcripts), mRNA editing (mRNA sequence is altered)

b. **Post translational modifications**- Proteins are modified (e.g., phosphorylation, glycosylation) after translation. There are about 200 different such modifications. Some polypeptides are edited and due to localization of proteins in cells proteins may be different.

Information sources

Full form	National Center for Biotechnology Information
Established in	1988
Parent Organizatio	National Library of Medicine (NLM), NIH
Function	To develop and maintain advanced information systems for molecular biology, and to make biomedical data freely accessible. Provide data retrieval systems and computational resources for analysis of biological data.
Website	https://www.ncbi.nlm.nih.gov

Major resources at NCBI

Category	Resource(s)	Description
1. Sequence Databases	GenBank, RefSeq, Nucleotide Protein	DNA, RNA, and protein sequences (raw and curated)
2. Sequence Analysis -Similarity Search Tools	BLAST, ORF Finder, Primer-BLAST	Tools for sequence comparison, primer design, and ORF detection
3. Gene, Chromosome & Genome Resources	Gene, Genome, Assembly	Gene-level data, genome assemblies, and mapping
4. Variation & Genotype	dbSNP, dbVar	Genetic variants, clinical relevance, and genotype-phenotype associations
5. Gene Expression & Functional Data	GEO, dbEST	Transcriptomics and functional genomics data
6. Chemical & Structure Data	PubChem	3D molecular structures and chemical information
7. Literature Resources	PubMed, PubMed Central (PMC)	Biomedical literature and full-text article access

Database retrieval tools

Tool	Purpose / Function
Entrez	Integrated database retrieval system- literature, sequences and structures. Gives comprehensive information on a given biological question.
Taxonomy Browser	Provides information on taxonomic classification of various species. It contains information of more than 79,000 organisms.
Locus Link	Carries information on the official gene names and other descriptive information about genes. It gives information on homologous genes.

BLAST family of Search Tools

Basic Local Alignment Search Tool (BLAST) is used for comparing biological sequences to existing database for-

- ***Identifying unknown sequences***- *By comparing the query sequence to the database*
- ***Finding homologous genes or proteins***- *By comparing the percentage of sequence similarity with other species for the same gene*
- ***Annotating genes***- *Labelling different parts of the sequences as exons, introns, ORF, etc.*
- ***Studying evolutionary relationships*** - Determining ancestral relationship

Principle involved in BLAST-

- A query sequence is compared with sequences in the database using substitution matrices (same or similar amino acids are scored high and chemically different amino acids are scored lower)
- Top scoring matches are ranked according to similarity due to ancestral relationship or due to random chance. These criteria can be changed by the user.
- True matches are examined thoroughly with other details accessible through Entrez and other tools available at NCBI.

Homologous and Paralogous sequences

Homologous	**Paralogous**
Genes that have common ancestry	Gene that have evolved due to duplication event
Same or different species	Usually within the same species or genome
May have similar function	May evolve new functions
Human vs. mouse insulin gene	Human haemoglobin α and β subun

Resources of gene level sequences

UniGene- database generated to manage redundant EST into a cluster that belongs to "one" gene.

HomoloGene- database of orthologs and homologs for several organisms like human, mouse, rat, zebrafish and cow genes represented in UniGene and Locus Link. It is easy to infer homologous relations using this database.

RefSeq- curated database of mRNAs and proteins of organisms like humans, mice and rats. The data is used to design gene chips and describe the sequence features of the human genome.

Database	**URL**	**Description**
GenBank	ncbi.nlm.nih.gov/genbank	Comprehensive nucleotide sequence repository from NCBI
ENA (EMBL-EBI)	ebi.ac.uk/ena	European Nucleotide Archive; international sequence sharing

Database	URL	Description
RefSeq	ncbi.nlm.nih.gov/refseq	Curated reference sequences for genes transcripts, and proteins
NCBI Gene	ncbi.nlm.nih.gov/gene	Gene-specific data including sequence function, and expression
PDB	rcsb.org	3-D structure of proteins
Ribosomal RN database	rnacentral.org/expert-databas s	rRNA subunit sequence
UniProt	uniprot.org	Protein sequences and functional information linked to gene data
UniGene	Database has been retired by NCBI	
Locus Link		Provides an integrated querying and cross-referencing system to facilitate movement from one source to another.
HomoloGene	Redirects to ncbi.nlm.nih.gov/datasets/ge	All genome related data of a taxon
PALI		Phylogenetic analysis and alignment o proteins

Curator- A curator at NCBI is responsible for checking-

- Newly submitted data ensuring all mandatory information has been provided.
- Biological features are adequately described
- Conceptual translations of any coding region obey known translation rules.

This process is called curation.

Analysis using Bioinformatics tools

Raw Information or sequence information of nucleotides can be processed and identified into genes, the protein that can be encoded and their function, the regulatory sequences and phylogenetic relationship.

1. Genes – Gene prediction can be done by using GeneMark- bacterial gene and GENSCAN for eukaryotes.
2. Proteins – Protein sequences can be inferred from predicted genes by using computer programs.
3. Regulatory sequences – Regulatory sequences can be identified using bioinformatics tools.
4. Inferring phylogenetic relationships – Multiple sequences alignment, calculation of evolutionary distance and constructing phylogenetic trees.
5. Making a discovery – Using bioinformatics tools and databases, functions of unknown genes can be predicted.

Solved Questions

Multiple Choice Questions

1. Which of the following is true about Huntington disease?

I. It is caused by mutation in HTT (Huntingtin) gene

II. Its inheritance is autosomal recessive

III. Location of HTT gene is chromosome 4 (4p16.3)

IV. Mutation is CAG repeats more than 35 times

a. I, II and III

b. II, III and IV

c. I, II and IV

d. I, III and IV

2. Nick Translation was developed by-

a. Rigby and Paul Berg in 1977

b. Craig Venter in 1990

c. Thomas and H Winkler in 1920

d. Thomas Roder in 1986

3. Which of the following is not a correct statement regarding sharing of their working DNA by different species?

a. Estimated difference between human and chimpanzee genomes is only 1 to 3%

b. Humans and mouse share about 97.5% of working DNA

c. **Genes in these organisms have not changed much since the common ancestor about 1000 million years ago**

d. Genes in these organisms have not changed much since the common ancestor about 100 million years ago

4. You have a sequence of unknown origin. Which of the following tools will you use to identify it?

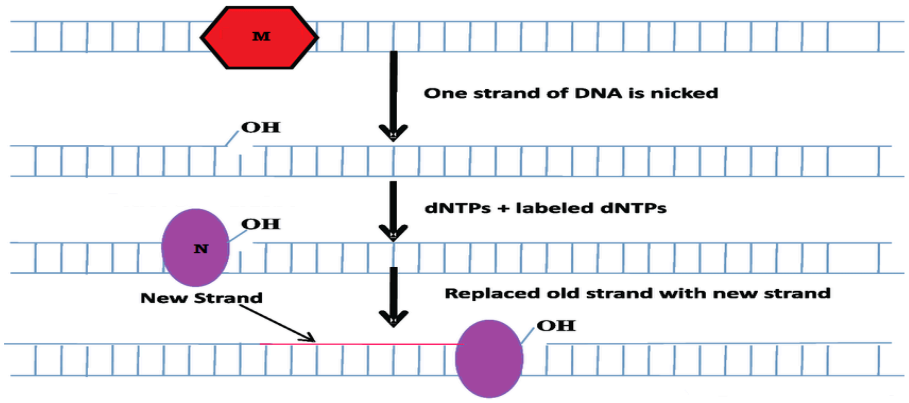
a. UniGene

b. BLAST

c. PALI

d. PDB

5. Which of your friends A, B, C and D have identified the diagram and labels M and N correctly?



a. Process- Nick translation, M- DNase I, N- DNA Pol I

b. Process- Dideoxy Chain Termination, M- DNase I, N- DNA Pol I

c. Process- Nick translation, M- DNA Pol I, N- DNase I

d. Process- Dideoxy Chain Termination, M- DNA Pol I, N- DNase I

6. Why is it not clear how to count genes for predicting the number of genes in an organism?

I. There are overlapping genes, splice variants that do not provide a clear picture of the genes.

II. There is no correlation between the complexity of organisms and number of genes.

III. The genome size of an organism and the part of the genome that encodes for protein are related.

IV. There are about 19000 genes in worms and 20000 to 25000 genes in humans.

a. I and II b. II and III c. III and IV d. IV and I

7. CML is caused due to –

a. Reciprocal translocation between chromosome 9 and 22

b. Formation of Philadelphia chromosome and extra-long chromosome 9

c. Fusion of abl-bcr gene

d. All of these

8. ENTREZ is a _____ tool

a. Data submission tool **c. Database retrieval tool**

b. Literature search tool **d.** SNP mapping tool

9. Which of the following is not a Mendelian trait-

a. Cystic Fibrosis **c.** Huntington disease

b. Migraine **d.** All of these

10. Which of the following is a Mendelian trait-

a. Cystic Fibrosis **c.** Alzheimer's disease

b. Migraine **d.** All of these

11. Which of the following is not an application of microarray technology?

a. Study of tissue specific genes

b. Study of gene defects in a disease

c. Cellular responses to environmental changes

d. Study of protein expression and its function

12. Which of the following is the correct definition of structural proteomics?

a. Study of Protein 3D structures and complexes present in a cell or organelle.

b. Study of protein activity in molecular networks in a cell like nuclear pore complex, interactions, and role in cellular processes.

c. Study of protein abundance and expression levels under different conditions or samples.

d. All of these

13. Which of the following is the correct definition of expression proteomics?

a. Study of Protein 3D structures and complexes present in a cell or organelle.

b. Study of protein activity in molecular networks in a cell like nuclear pore complex, interactions, and role in cellular processes.

c. Study of protein abundance and expression levels under different conditions or samples.

d. All of these

14. Which of the following is the correct definition of functional proteomics?

a. Study of Protein 3D structures and complexes present in a cell or organelle.

b. Study of protein activity in molecular networks in a cell like nuclear pore complex, interactions, and role in cellular processes.

c. Study of protein abundance and expression levels under different conditions or samples.

d. All of these

Following questions consist of two statements – Assertion (A) and Reason (R). Answer these questions selecting the appropriate option given below:

A. Both Assertion and Reason are true and the reason is the correct explanation of the assertion

B. Both Assertion and Reason are true but the reason is not the correct explanation of the assertion

C. Assertion is true but Reason is false

D. Both Assertion and Reason are false

15. **Assertion**- Cystic fibrosis is caused by single gene mutation which follows Mendelian inheritance.

Reason- Cystic fibrosis is caused by the mutation in the CFTR gene located on chromosome 7.

Ans- A

16. **Assertion**- Two sequences are said to be homologous if they have similarity due to duplication of genes.

Reason- Two sequences are said to be paralogous if they have similarity due to common ancestry of genes.

Ans- C

17. **Assertion**- FISH is a technique used to study gene expression in a cell.

Reason- Nick translation is used to introduce colour in cDNA.

Ans- D

18. **Assertion**- It is estimated that the difference between human and chimpanzee genome is only 1 to 3 %.

Reason- The genomes have not changed much since these two organisms shared a common ancestor about 100 million years ago.

Ans- A

19. **Assertion**- Proteome of a cell is dynamic.

Reason- Large scale study of proteome is called proteomics.

Ans- B

20. **Assertion**- Gene Chips can be designed using the RefSeq database.

Reason- RefSeq is a curated database that contains information on tRNA of humans.

Ans- C

21. **Assertion-**NCBI has many sources and information on biological molecules, pathways, sequences, structure etc.

Reason- NCBI was created in 1988 to provide the tools and programs to make this information available to the public.

Ans- A

22. **Assertion**- Curator checks the raw sequence that is submitted to the NCBI database.

Reason- All the mandatory information that is submitted about the sequence to the database is checked by the curator

Ans- B

23. **Assertion**- Gene prediction of bacterial genes can be done by GENSCAN.

Reason- Gene prediction of eukaryotic genes can be done by GeneMark.

Ans- D

24. **Assertion**- No. of genes are not equal to the number of proteins.

Reason- mRNA undergoes many post-translational changes and polypeptide undergoes many post-transcriptional changes.

Ans- C

Short Answer Type Questions

25. What are the different types of molecules on which sequence data is obtained and deposited in a database?

Proteins, DNA and RNA

26. How are genes linked to diseases? Give 2 examples.

Mutated genes or changed gene functions may manifest as disease or increase susceptibility to the disease.

Example- CFTR gene- Cystic Fibrosis and HTT gene- Huntington's disease

27. Observe the flow chart given below and answer the following questions.

a. Identify B and D?

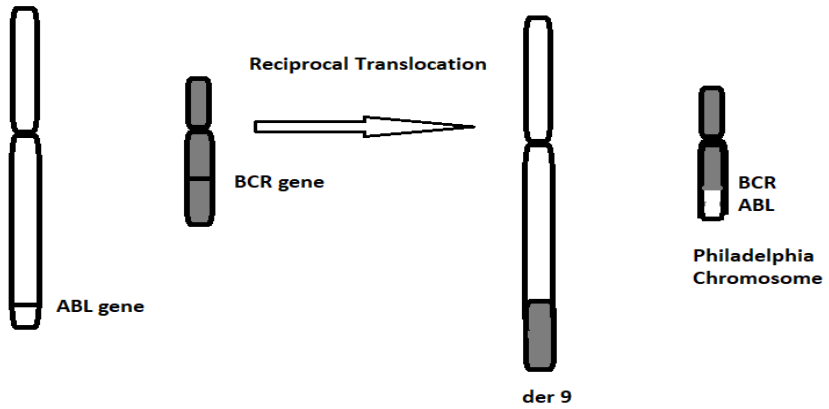
b. Analysis of mRNA does not provide an accurate reflection of the protein content in a cell. Why?

Ans- a- B- post transcriptional modification, D- post translational modifications

b- Analysis of mRNA does not provide an accurate reflection as the polypeptide produced may undergo many different post translational modifications.

28. Illustrate reciprocal translocation to show the formation of the Philadelphia chromosome.

Ans-



29. How is FISH used to determine the efficacy of a drug in case of CML?

CML is caused due to reciprocal translocation between chromosome 9 and 22 and formation of the Philadelphia chromosome. Using FISH, Chromosome 9 is painted red and chromosome 22 is painted green. Philadelphia chromosome shows yellow colour. Using FISH the number of cells having yellow colour before and after the administration of drug can be studied.

30. What is proteomics? How can we benefit from proteomics?

Ans- Proteomics is the large-scale study of proteins, including their expression, structures and functions.

Advantages of Proteomics-

a. Understanding protein structure and their functions in cell/ tissue/ organism

b. Identification of target for treatment of diseases and diagnosis

c. Study of networks of proteins and predict functions of newly discovered proteins

d. Disease mechanisms and possible treatment

31. Name some database retrieval tools. What is their purpose?

Tool	Purpose / Function
Entrez	Integrated database retrieval system- literature, sequences and structures. Gives comprehensive information on a give biological question.
Taxonomy Browser	Provides information on taxonomic classification of various species. It contains information of more than 79,000 organisms.
Locus Link	Carries information on the official gene names and other descriptive information about genes. It gives information or homologous genes.

Long Answer Type Questions

32. What is BLAST? Describe the principles that underlie BLAST search.

Ans- Basic Local Alignment Search Tool (BLAST) is used for comparing biological sequences to existing database for-

- Identifying unknown sequences- By comparing the query sequence to the database
- Finding homologous genes or proteins- By comparing the percentage of sequence similarity with other species for the same gene
- Annotating genes- Labelling different parts of the sequences as exons, introns, ORF
- Studying evolutionary relationships - Determining ancestral relationship

Principle involved in BLAST-

- A query sequence is compared with sequences in the database using substitution matrices (same or similar amino acids are scored high and chemically different amino acids are scored lower)
- Top scoring matches are ranked according to similarity due to ancestral relationship or due to random chance. These criteria can be changed by the user.
- True matches are examined thoroughly with other details accessible through Entrez and other tools available at NCBI.

33. Using microarrays one can identify the genes expressed differently in normal vs cancer cell types. Explain.

Ans- Microarray is a tool used to study the expression of many genes at once by measuring the amount of labelled cDNA probes (reverse transcribed from mRNA) bound to a gene chip or comparing the gene expression of two different types of cells.

Principle- the principle of microarray technology is based on complementary base pairing and hybridization — the ability of complementary DNA strands to bind, or hybridize, to each other. There are several small spots on a glass / silicon base each containing specific DNA fragments representing a gene. These DNA fragments can bind and hold the labelled probes that are applied to the gene chip by hybridization.

Diagram: refer above

34. What are SNPs? Explain with an example. How many SNP sites are present in the human genome? How do SNP mapping of patients help physicians in determining the effectiveness of a prescribed drug?

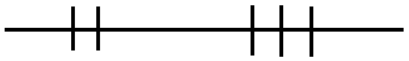
Ans- SNP- Single nucleotide polymorphism is change in a single nucleotide at a given location in different individuals

Example- AT**G**CGT

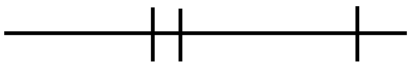
AT**C**CGT

1.3 to 3.2 million sites in human genome

Patients showing a positive SNP pattern may be given the drug and patients showing a negative pattern may not be given the drug.



Patient with positive response



Patient with negative response

SNP mapping of patients with different responses to a drug

35. Suggest one possible way to analyze a given sequence using bioinformatics.

Ans- Determine if the sequence is nucleotide or protein sequence.

Sequence search with BLAST to compare with known sequences to determine its origin.

Gene Prediction with GeneMark or GENSCAN to identify whether the sequence is a gene. If it is a gene sequence then the translated protein sequence can be inferred from it.

Regulatory sequences can also be determined by using bioinformatics tools.

Phylogenetic relationships can also be determined and phylogenetic trees can be constructed.

If the gene has a previously unknown protein sequence, then the structure and functions of the protein can be determined using bioinformatics.

Case Based Question

36. **Study the given table and answer the following questions**:

Table 1. Genome size and gene predictions between several organisms.

Organism	No. of chromo somes	Genome size in base pairs	The Number of Predicted genes	Part of the genome that encodes for protein
Bacteria *Escherichia coli*	1	500,000	5000	90%
Yeast *Saccharomyces cerevisiae*	16	12,068,000	6340	70%
Worm *Caenorhabditis elegans*	6	100,000,000	19,000	27%
Fly *Drosophila melanogaster*	4	175,000,000 - 196,000,000	13,600	20%
Weed *Arabidopsis thaliana*	5	157,000,000	25,498	20%
Human *Homo sapiens*	23	3,000,000,000	20,000 - 25, 000	< 5%

a. **What is the trend observed in the percentage of the genome that encodes protein in the table given?**

Ans- The percentage of the genome that encodes protein decreases as the size of the genome increases.

b. Why is the in-silico gene prediction considered unreliable?

Ans- It is not clear how to count the number of genes when many splice variants are observed in different tissues from the same segment of gene.

c. How is computational biology used to identify a gene? How does this become a limitation?

Ans- In computational biology gene prediction can be done by creating an algorithm with our existing knowledge of nucleotide structure of genes like promoters, ORFs, start codons, exons, introns, stop codons.

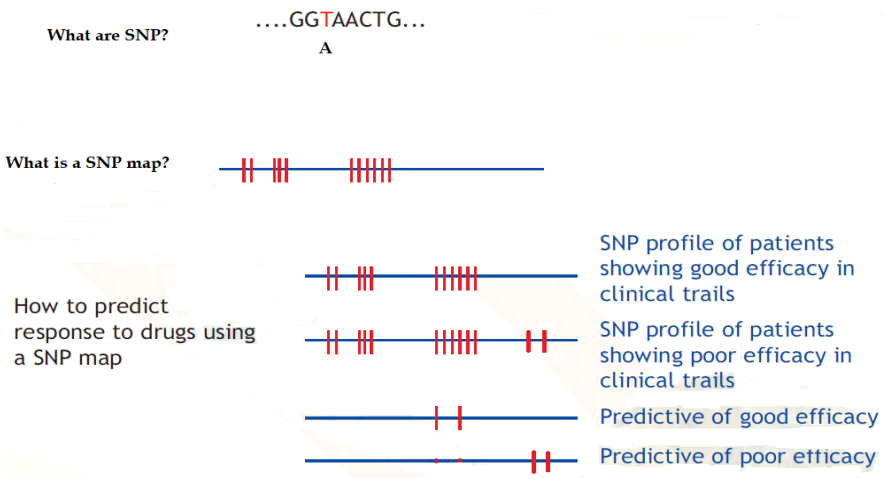
Limitations in these algorithms are due to overlapping genes and splice variants.

OR

Why is the number of genes in humans so low as compared to other organisms in the gene prediction? Why is this number considered low when compared to other organisms?

Ans- The number of genes is low as compared to other organisms due to presence of overlapping genes and splice variants. It is considered low as the complexity of the organisms does not translate into a greater number of genes.

37. The below figure depicts a process used in diagnosis of a disease.



a. **Write a sequence to show a possible SNP of the given sequence** GG**T**AACTG

Ans- GGAAACTG

b. **Mention any one beneficial variation in the human genome?**

Ans- Simple deletion in CCR5 leads to resistance to HIV infection

c. **How do physicians use SNP maps to predict drug efficacy?**

Ans- Physicians can check the SNP map of the patient with the SNP maps of those with positive response during clinical trials. If it matches then the patient can be given the drug otherwise not.

OR

The following are the estimated differences between different coding regions of the genome of different species. What can be inferred about ancestors from these differences? Give reason for your answer.

i. Species A and B – 2.5 % - shares a recent common ancestor. Greater similarities

ii. Species C and D – 10 %- share a distant common ancestor. Lesser similarities

UNIT- 6

Cell Culture and Genetic Manipulation

Chapter 1: Microbial Cell Culture

Microbial Growth Requirements: Water, Energy Source, Carbon Source, Nitrogen Source, Oxygen, Mineral Elements

Scale of Culture

a. Small-scale (Laboratory)

Nutritive Media Types:

- Synthetic (Pure chemicals, known composition)
- Semi-synthetic (Complex components, e.g., peptone)

Examples: Nutrient Broth, Trypticase Soya Broth (TSB), BHI etc

b. Large-scale (Industrial)

- Nutrient Sources
- Economical & Readily Available
- May require pretreatment

General Considerations for all Growth Media Selection

- Maximize product/biomass yield per gram of substrate.
- Consistent quality and year-round availability.
- Minimize problems during preparation and sterilization.
- Minimize problems during production (aeration, agitation, extraction, purification).

Microbial Culture Sterilization Procedures

1. Small-scale (Laboratory, 100-1000 ml flasks)

- Method: Autoclave
- Conditions: 121°**C,** 15 psi, 15-20 minutes.

2. Large-scale (Industrial Fermentation)

A. Medium Sterilization - Medium may be sterilized *in situ* (in the fermentor) or in a separate vessel

- Method: Steam

B. Fermentor Sterilization (if medium sterilized separately)

- Method: Steam
- Application: - Passing steam through jacket/coils.
- Sparging steam into vessels through entries.
- Conditions: 15 psi, 20 minutes

Microbial Culture Methods

1. Batch Culture

- Closed system with initial limited nutrients and one time Inoculation with bacteria.
- Growth phases: Lag, Log (Exponential), Stationary (growth = 0), Decline.
- Nutrients consumed, metabolites accumulate as culture proceeds.
- Environment changes over time.

Example: Culturing microbes in an ordinary flask

2. Fed-batch Culture

- Batch culture with continuous/sequential addition of fresh medium.
- No removal of growing culture.
- Substrate concentration remains relatively constant.
- Cell density increases over time.
- Volume in culture vessels increases over time.

3. Continuous Culture

- Continuous supply of microbial growth/products.
- Growth medium with one limited nutrient.
- Fresh medium is added as the limited nutrient is about to be exhausted.
- Equal volume of culture removed (overflow device).
- Cell growth at a particular rate for an extended period.
- Steady state: Cell growth and substrate consumption at a fixed rate.
- Constant cell, metabolite, and nutrient concentrations at steady state.
- Suitable for production of biomass or metabolites.

Types:

a. Chemostat: maintains Constant chemical environment

b. Turbidostat: maintains Constant cell concentration

Microbial Growth Measurement and Kinetics

Microbial Growth: Orderly increase in all chemical components. Measured by cell mass or cell number. Characterized by doubling time (td).

General Growth Patterns: Bacteria (binary fission), Yeast (budding), Mold (chain elongation & branching), Viruses (intracellular, irregular).

Methods for Measuring Cell Growth:

- **Dry Weight:** Measure dry mass of cells in a fixed volume.
- **Spectrophotometry (Absorbance/Turbidity):** Measures light scattering proportional to cell concentration. Requires a standard curve.
- **Other Methods:** Wet weight, turbidity measurements (related to absorbance), ATP measurement, viable plate count (CFU), Coulter counter (electronic cell counting).

Microbial Growth Kinetics (using bacterial binary fission as example):

- **Generation Time (Doubling Time, td):** Time required for a cell to divide and the population to double.
- **Exponential/Logarithmic Growth:** Cell number increases exponentially with each division.
- Microbial growth in the exponential phase is measured related to either biomass or cell number.
- Specific growth rate is an index of rate of growth of the cells in a particular environment. Specific growth rate is characteristic of the microorganism and is a function of the growth environment including temperature, pH, medium composition and levels of dissolved oxygen.

Downstream Processing

Downstream Processing: Recovery and purification of desired metabolites after fermentation.

General Steps:

1. **Cell Separation:** Removing microbial cells (biomass/pellet) from the broth (centrifugation or ultrafiltration). Settling with/without flocculants can also be used.

2. **Cell Disruption/Broth Concentration:**

   - **Intracellular product:** Cell disruption needed.
   - **Extracellular product:** Broth concentration is processed.

3. **Initial Purification:** Preliminary removal of impurities from the metabolite.

4. **Metabolite-Specific Purification:** High-degree purification of the target metabolite.

5. **Polishing:** Final concentration and formulation to achieve 98-100% purity.

Key Operations:

- **Cell Separation:** Centrifugation, ultrafiltration, settling (± flocculants). Ultrafiltration retains large particles (cells) using fine pore membranes (<0.5 μm).
- **Metabolite Recovery (Clarified Broth):** Precipitation, solvent extraction, ion exchange chromatography.
- **Concentration & Purification:** Various downstream operations available.

Efficiency: Fewer steps are preferred to minimize production cost and maximize yield.

Strain Isolation:

Enrichment Technique: Provide growth conditions (e.g., temperature) and nutrients that favour growth of desired microbes. Repeated subculturing improves growth of desired organisms

Screening method for desired properties in organisms.

1) Biochemical: Antibiotic producer: Agar plate with target bacterium.

2) Immunological methods: Detection using specific antibodies.

3) Molecular biology: Use of probes for specific product genes (adapted to robotic automation resulting in enormous throughput screening of microbes for newer / novel molecules.

Strain Improvement:

Done to enhance production of desired molecules to economically viable levels in an organism.

SL. NO.	Mutant Selection	Genetic Engineering
1	Oldest method of strain improvement	Add totally new properties/capabilities to microorganisms
2	Expose strain to Chemical mutagen (e.g., NTG) or Physical mutagens (e.g., UV rays	Create recombinant strains
3	Select mutants with improved characteristics	Manipulation to: Synthesize/secrete enhanced biomolecules, Produce novel compounds, Utilize cheaper substrates,
4	Often requires multiple successive mutations	Examples of human proteins produced in microorganisms
5	Example: Penicillin production: *Penicillium chrysogenum* mutant produces 100x more penicillin than *P. notatum*	Recombinant human insulin (Humulin), Hepatitis B surface antigen

Importance of Preservation:

- Once a desired microbial strain is obtained, proper preservation is crucial for future use.
- Improper preservation can lead to:
  - Loss of viability (the strain dies).
  - Decline in the production of the target product.

Methods of Preservation:

1. **Storage on Agar:**
   - Cultures are grown on agar slants or stabs.
   - Stored at temperatures ranging from 5°C to -20°C.
   - Requires periodic sub-culturing (transferring to fresh media) approximately every 6 months to maintain viability.
   - Sub-culturing interval can be extended up to 1 year by covering the culture with sterile mineral oil, which reduces desiccation and oxygen availability.
2. **Storage in Liquid Nitrogen (Cryopreservation):**
   - A cryoprotective agent, such as glycerol (at a concentration of 10-30%), is added to the culture medium to protect cells from damage during freezing.
   - The mixture is dispensed into sealed ampoules.
   - Ampoules are rapidly frozen and stored in liquid nitrogen at extremely low temperatures (-176°C to -196°C).
3. **Lyophilisation (Freeze-Drying):**
   - Involves two main steps:
     - **Freezing:** The microbial culture is rapidly frozen.
     - **Drying under Vacuum:** The frozen culture is subjected to a high vacuum, causing the ice (cell water) to sublimate (transition directly from solid to gas).
   - The resulting lyophilised culture is a dry powder that can remain viable for a long period, typically 5-10 years or even longer.

<u>Metagenomics for Novel Molecule Discovery</u>

Metagenomics: Direct utilization of collective genomes (metagenome) from an environmental niche (soil, water, gut, etc.) to identify novel molecule-synthesizing genes.

Key Aspects:

- Analyses DNA from both culturable and non-culturable microbes.
- Bypasses the need for individual microbial culturing.

Process:

1. Extract collective DNA.
2. Restriction digestion.
3. Fragment cloning.
4. Screening clones for novel molecules.
5. Large-scale production (fermentation) of positive clones.

Advantages:

- Wider exploration of microbial resources.

- Access to genes from uncultured microorganisms.
- Potential to discover novel molecules for human use.

Applications of microbial cell culture

Microbial culture has immense potential for the production of very useful compounds. In general, microbial cultures can be exploited primarily in six different ways for the production of metabolites. They are listed below

- Production of whole microbial cells (food, vaccine).
- Production of primary metabolites (acids, alcohol)
- Production of secondary metabolites (antibiotics)
- Biotransformation reactions (enzymatic, steroid)
- Exploitation of metabolism - Microbial leaching, waste treatment.
- Recombinant proteins - therapeutic proteins gene delivery vectors/DNA.

The main areas of consideration for safety aspects specific to biotechnology are:

- Pathogenicity: Potential of modified living organisms and viruses to infect humans, animals and plants to cause diseases.
- Toxicity and allergy associated with microbial production.
- Other medically relevant effects like increasing the environmental pool of antibiotic-resistant microorganisms.
- Problems associated with the disposal of spent microbial biomass.
- Safety aspects associated with contamination, infection or mutation of process strains.

Solved Question Paper

MULTIPLE CHOICE QUESTIONS

1. At a steady state in continuous culture, what remains constant?
(a) Only cell concentration
(b) Only the concentration of metabolites
(c) **The concentration of cells, metabolites, and other nutrients**
(d) Only the growth rate

2. What is the purpose of the overflow device in a continuous culture system?
(a) To prevent contamination.
(b) **To maintain a constant volume of culture.**
(c) To increase the rate of nutrient addition.
(d) To measure the growth rate of the culture.

3. What is the relationship between absorbance and cell concentration in a spectrophotometer reading?
(a) Inversely proportional (b) Unrelated
(c) **Directly proportional** (d) Exponentially related

4. A researcher measures the absorbance of an unknown microbial sample at a specific wavelength. How can they determine the cell concentration of the sample?
(a) **By comparing the absorbance to a standard graph of absorbance versus cell concentration.**
(b) By multiplying the absorbance by a constant factor.
(c) By dividing the absorbance by a constant factor.
(d) By subtracting the absorbance from a known value

5. Which of the following best describes mutation selection as a method of strain improvement?
a) A process that uses only physical mutagens to enhance strain characteristics.
b) A method that relies solely on selecting naturally occurring mutations.
c) **A technique involving the exposure of strains to mutagens and the selection of improved mutants.**
d) A process that always yields desired results in a single mutation step

6. What characteristic of the expression vector is emphasized in maximizing protein production?
a) Low replication rate
b) Low copy number
c) **High copy number and stability**
d) Instability in the host

7. Why might a regulatory switch be used in the context of foreign gene expression?
a) To permanently deactivate the foreign gene.
b) **To control the timing of recombinant protein production.**
c) To enhance the host's immune response.
d) To increase the bacteria's reproduction rate.

8. Which of the following best defines "metagenome"?
a) The collection of culturable microbes in a lab
b) **The total collection of microbial DNA from a specific environmental sample**
c) The DNA of only culturable microbes
d) A type of genome sequencing technique

9. What is the ultimate aim of screening clones in metagenomics research?
a) To find the most common microbes in an ecosystem
b) **To identify and produce molecules with enhanced characteristics for large-scale use**
c) To isolate pure cultures of traditional microbes
d) To improve laboratory culturing techniques for rare species

10. What is the primary purpose of culture collection centres?
a) To sell cultures to commercial entities
b) **To safely maintain and provide access to biological cultures**
c) To conduct research on genetic modifications
d) To train scientists in microbiology

11. Why are culture collection centres governed by stringent rules and regulations?
a) To ensure cultures can survive longer
b) To prevent contamination of cultures
c) **To protect the intellectual property rights of depositors**
d) To promote international collaboration on research

12. How is the fermentor typically sterilized during large-scale fermentations?
A) By boiling the contents inside

B) By using chemical sterilant
C) **By passing steam through its jacket or coils**

D) By exposing to ultraviolet light

13. What is the common pressure used during sterilization in an autoclave for laboratory-sized flasks?
A) 10 psi B) **15 psi** C) 20 psi D) 25 psi

14. In large-scale fermentation processes, what is the main challenge presented in sterilizing culture medium or substrates?
A) Ensuring the nutrient medium stays cool
B) **Sterilizing thousands of liters at once**
C) Adding nutrients directly to the fermentor
D) Avoiding contamination from air exposure

15. What are synthetic media?
A) Media with complex natural components.
B) **Media made from pure chemicals with known compositions**
C) Commercially available media
D) Media containing only carbohydrates

16. Which of the following is NOT a type of semi-synthetic media?
A) Nutrient broth B) Trypticase soya broth (TSB)
C) Brain heart infusion (BHI) broth D) **Glucose broth**

17. Which of the following is commonly used as a carbon source in microbial culture?
A) Ammonium salts B) Corn steep liquor
C) **Cane molasses** D) Urea

18. Why are growth factors needed in some microbial cultures?
A) They increase the pH of the medium.
B) **They provide essential nutrients that microbes cannot synthesize.**
C) They reduce the metabolic rate of microbes.
D) They act as preservatives in culture media.

19. What is the main problem caused by foaming in microbial processes?
A) It enhances nutrient absorption.
B) It facilitates protein synthesis.
C) **It hinders the diffusion of oxygen.**
D) It improves the texture of the medium.

20. Which of the following substances is NOT mentioned as an antifoam agent?
A) Olive oil
B) Silicones
C) **Glycogen**
D) Sunflower oil

21. What type of water is preferred for culturing microbes in the laboratory?
A) Tap water
B) **Single or double distilled water**
C) Mineral water
D) Reverse osmosis water

22. What should be considered when assessing water for large-scale microbial culture?
A) Temperature and colour
B) **pH and dissolved salts**
C) Taste and odour
D) Availability and cost

23. What is the primary purpose of continuous culture in microbial growth?
A) To minimize nutrient consumption
B) **To obtain a constant supply of microbial growth and/or products**
C) To increase the time for microbial cell division
D) To achieve maximum nutrient depletion

24. In continuous culture, what happens when the limited nutrient is nearly exhausted?
A) The culture vessel is emptied
B) The growth rate accelerates
C) **Fresh medium is added to maintain growth**
D) The system automatically shuts down

25. What device is used in a continuous culture system to maintain the culture volume?
A) Incubator
B) **Overflow device**
C) Sedimentation tank
D) Filtration system

26. In a chemostat, which condition is maintained constant?
A) Temperature
B) Nutrient concentration
C) **Chemical environment**
D) Cell concentration

27. What is the main characteristic of a turbidostat in a continuous culture system?
A) Maintains a constant rate of nutrient feed
B) Promotes maximum biomass accumulation
C) Increases nutrient supply over time
D) **Ensures constant cell concentration**

28. What does the term “steady state” imply in continuous culture?
A) **The rate of cell growth and substrate consumption is fixed**
B) All nutrients are completely consumed
C) Growth rate of cells is increasing
D) The culture vessel is emptied regularly

29. During steady state operation, which of the following remains constant?
A) **Concentration of cells, metabolites, and nutrients**
B) Temperature and pressure
C) Volume of the reactor
D) Rate of nutrient exhaustion

30. Why is continuous culture particularly suitable for the production of microbial biomass or metabolites?
A) It maximizes nutrient waste
B) It allows for indefinite biomass growth
C) **It can maintain a constant production rate over time**
D) It encourages rapid fluctuations in culture conditions

31. In which of the following scenarios would a continuous culture be less effective?
A) When consistent microbial biomass is required
B) **When there is a high variability in nutrient availability**
C) If steady metabolite production is desired
D) When operational costs are minimized

32. What is a key operational advantage of using a continuous culture system?
A) Reduced maintenance requirements
B) Ability to recycle waste products
C) **Constant production environment**
D) Increased chance of culture contamination

ASSERTION – REASON QUESTIONS

a) Both assertion and reason are true and the reason correctly explains the assertion.

b) Both assertion and reason are true but the reason does not explain the assertion.

c) Assertion is true and reason is false.

d) Both assertion and reason are false.

e) Assertion is false and reason is true.

1. Assertion: When a eukaryotic gene is expressed in a prokaryotic host, the non-coding regions (introns) of the eukaryotic gene are not removed for proper protein synthesis.

Reason: Prokaryotic cells have the cellular machinery (spliceosomes) necessary to process and remove introns from pre-mRNA.

2. Assertion: Expressing a eukaryotic gene in a prokaryotic host necessitates the use of reverse transcription of mRNA into cDNA.

Reason: cDNA, synthesized from mature mRNA, contains only the coding sequences (exons) of the eukaryotic gene, devoid of introns.

3. Assertion: Studying a metagenome provides a more comprehensive understanding of the microbial diversity and potential functions within an ecosystem compared to traditional culture-based methods.

Reason: Metagenomics includes the genetic contributions of the vast majority of microbes that are difficult or impossible to grow in laboratory conditions.

4. Assertion: The term "metagenome" refers to the combined genetic material of all microorganisms within a specific environmental niche.

Reason: Metagenomics bypasses the need for individual culturing of microbes and instead focuses on the total DNA extracted from the environmental sample.

5. Assertion: The specific growth rate of a bacterial species will remain constant regardless of the surrounding environmental conditions.

Reason: Specific growth rate is solely determined by the genetic makeup of the microorganism and is independent of factors like temperature and nutrient availability.

6. Assertion: The specific growth rate of a microorganism is a measure of how quickly its population increases per unit of biomass under defined conditions.

Reason: Specific growth rate reflects the intrinsic growth capacity of a particular microorganism in all environments.

7. Assertion: During the stationary phase of batch culture, there is no net increase in the number of viable cells.

Reason: In the stationary phase, the rate of cell growth is balanced by the rate of cell death, resulting in a constant population size.

8. Assertion: The environment within a batch culture remains constant throughout the entire growth period.

Reason: As microorganisms grow in batch culture, they consume nutrients and release metabolic byproducts, altering the composition of the growth medium.

9. Assertion: Forced aeration is a technique used in small-scale bioreactors to supply oxygen and promote mixing.

Reason: Introducing air or oxygen into the culture vessel under pressure decreases the oxygen concentration in the liquid medium.

10. Assertion: Shake culture is a simple and effective method for achieving aeration and mixing in laboratory-scale microbial cultivation.

Reason: Placing flasks on shakers provides agitation, which enhances the transfer of oxygen from the air into the liquid medium.

ANSWERS

1	2	3	4	5	6	7	8	9	10
D	a	a	b	d	c	a	e	b	a

Very Short Answer Questions

1. Water is a major component in microbial cell culture. What should be considered while assessing its suitability at small scale and large-scale microbial culture?

Ans. When required for culturing microbes in the laboratory, single distilled or double distilled water would suffice. When assessing its suitability for large-scale microbial culture as in industry, we should consider the pH and dissolved salts.

2. A researcher is using baffle flasks for small scale microbial culture in the lab. Why is he preferring baffle flask over normal conical flask?

Ans. Baffle flasks have V- shaped notch or indentation in the sides of the flask. Such flasks are called baffle flasks. This improves the growth of the microbes by improving the efficiency of oxygen transfer due to increased turbulence of the agitated culture medium.

3. What is the capacity of a small scale biofermentor used in laboratories? Mention any two uses of such bioreactors.

Ans. Small scale fermentors of capacity 10 -100 liters are used in research laboratories. These are used by the scientists in research, to optimize various parameters for the growth of microbes. The laboratory scale fermentors are also used by scientists, to produce enough quantities of metabolites from microbes for research purposes.

4. A bioreactor plant has only impellers to enable aeration and mixing. What kind of bioreactor is the industrial unit using? Is it efficient for proper aeration and mixing? Why?

Ans. Stirred tank bioreactor. No, as forced aeration through spargers at the bottom is also required.

5. A scientist performing culture at his lab observes that a microbial strain is showing a very slow growth rate as compared to the standard graph. How is he measuring the growth?

Ans. By using spectrophotometric absorbance method.

Short Answer Questions

1. Expression of a foreign gene in a host can face several problems. Mention any three such problems and their respective solutions.

Ans.

SL. NO.	Problem	Solution
1	Non expression of foreign gene	Putting the gene under the control o a prokaryotic host gene's promoter
2	Low expression	Using strong inducible promoter and a high copy number expressio vector
3	Eukaryotic gene	Making cDNA through revers transcription.

2. Although *Saccharomyces cerevisiae* has been extensively studied and easy to grow, why is *Pichia pastoris* preferred for expression of eukaryotic foreign genes?

Ans. Product yields in *Saccharomyces cerevisiae* are relatively low at 1-5% of the total protein. Other yeasts like *Pichia pastoris* have a number of advantages: it has strong inducible promoters; it is capable of making post-translational modifications similar to those performed by human cells; downstream processing is simpler as Pichia does not secrete its own proteins into the fermentation medium**.**

3. Mention any three advantages of culture collection centres. Name one National and one International culture collection centre.

Ans. These centres safely maintain cultures for years. The cultures are also made available to prospective investigators. With the advent of modern biotechnology and the associated commercial and financial implications, the culture collection centres are governed by stringent rules & regulations to protect the intellectual property rights of the depositors.

National : MTCC (Microbial Type Culture a Collection and Gene Bank)

International: NCIB (National Collection of Industrial Bacteria, UK)

4. **Fill in the blanks:**

Sl. No	Microorganism	Product
1	Saccharomyces cerevisiae	----------
2	----------	Amylases
3	----------	Dextran

5. What are GRAS microbes? Mention any three specific safety aspects for microbial technology.

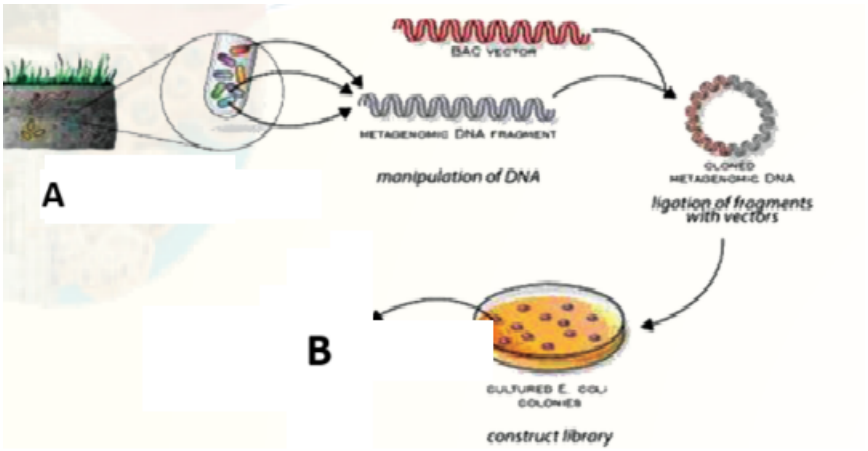
Ans. GRAS: (generally regarded as safe) microbes which are non-toxic, non allergic and non producers of secondary metabolites.

Microbial technology safety aspects:

- Potential of genetically modified organisms (GMO) or recombinant strains to infect humans, animals and plants cause diseases.
- Toxicity and allergy associated with the use of microbially produced biomolecules especially the recombinant molecules.
- Other medically relevant implications like increasing the environmental pool of antibiotic resistant microorganisms or transfer of antibiotic resistant genes.

Long Answer Questions

1. Answer the questions from the given diagram:



a) Identify A & B from the above diagram. Name the technique that is described in the diagram.

Ans: A: Isolation of DNA from environmental samples.

B: Expression of proteins and analysis.

Technique: Metagenomics

b) What is the need for BAC vectors in the technique?

Ans. The digested DNA fragments are larger in size and BAC has larger insert size with more stability than YAC.

c) What is the purpose of the technique and how is it better than routine culture-based experiments?

Ans. Metagenomics searches for novel genes producing novel products of therapeutic interests or beneficial purposes directly from environmental samples with the need for culture and isolation of specific microbes. It enables fishing out desired novel genes from non-culturable microbes too.

2. a) Calculate the generation time (doubling time or td) of a bacterial population in which the number of bacteria increases from 10^6 /ml to 10^{12}/ml during four hours of exponential growth.

b) What is the specific growth rate?

Ans. Specific growth rate is an index of rate of growth of the cells in a particular environment. In other words, specific growth rate is characteristic of the microorganism and is a function of the growth environment including temperature, pH, medium composition and levels of dissolved oxygen.

c) What is the relation between specific growth rate and doubling time?

Ans. A microbial strain with higher specific growth rate will have low doubling time and vice versa.

$td=0.693/\mu$

3. a) What is downstream processing? Why is it advisable to use a smaller number of steps in DSP?

Ans. A collective number of steps involving the isolation of pure form of a protein after biofermentation of a microbial strain. But it is always advisable to use a lesser number of steps to achieve the desired purity of the metabolite or product. This is because, more the number of steps involved, more will be the cost of the production and lower would be the yield.

b) Draw a flowchart to describe the isolation of streptomycin from *Streptomyces griseus*.

c) What are excipients?

Ans. Once the pure metabolite is obtained, a stabilized formulation is made using several ingredients known as excipients.

CASE BASED QUESTIONS

1. Metagenomics

A study of the metagenome of the microbial inhabitants of the Sargasso Sea, generated sequences of about a million genes and revealed whole classes of genes that were more diverse than could ever have been anticipated on the basis of studies of cultured organisms. Studies of a simple microbial community that lives in the extremely acidic water draining from metal mines demonstrated the potential of Metagenomics to dissect detailed interactions among microbial-community members. Metagenomics, however, is more than just large-scale sequencing. In function-based Metagenomics, millions of random DNA fragments in a library are translated into proteins by bacteria that grow in the laboratory. Clones producing "foreign" proteins are then screened for various capabilities, such as vitamin production or antibiotic resistance. This enables researchers to access the tremendous genetic diversity in a microbial community without knowing anything about the underlying gene sequence, the structure of the desired protein, or the microbe of origin. New antibiotics and resistance mechanisms have already been discovered using function-based Metagenomics.

1) Metagenomics involves

1. The study of meta genome
2. Fishing out of genes from the pool of culturable and non culturable genome
3. **Both 1 and 2**
4. Only 1

2) **Choose the correct sequence of the procedure of Metagenomics technique**

A. Isolation of DNA
B. Expression and analysis of proteins
C. Creation of DNA library
D. Restriction digestion

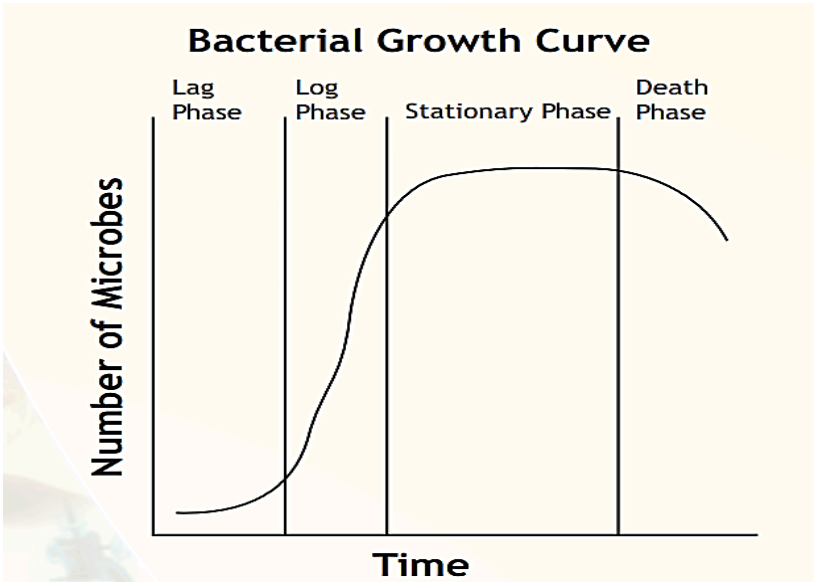
1. D B A C
2. A B C D
3. AC D B
4. **A D C D**

3) **What is the primary focus of metagenomics**

a) Analyze the genome of an organism
b) **Investigate DNA directly from environmental samples**
c) Analyze the mutation present in an organism
d) Determining protein structure in a community

2. Microbial growth kinetics

A clear understanding of microbial growth is necessary for proper utilization of biological processes for production of metabolites. In this section, the methods used for the measurement and quantitative evaluation of microbial growth will be discussed. There are four general patterns of microbial growth exemplified by bacteria, yeast, mold and viruses. All these microorganisms grow in different ways: bacteria grow by binary fission, yeast divide by budding, fungi divide by chain elongation and branching whereas viruses normally do not follow a regular growth pattern as they grow intracellularly in host cells.



Microbial growth kinetics, i.e., the relationship between the specific growth rate (μ) of a microbial population and the substrate concentration (s), is an indispensable tool in all fields of microbiology, be it physiology, genetics, ecology, or biotechnology, and therefore it is an important part of the basic teaching of microbiology.

A. Which of the following is the best definition of generation time in a bacterium?

1. the length of time it takes to reach the log phase
2. **the length of time it takes for a population of cells to double**
3. the time it takes to reach stationary phase
4. the length of time of the exponential phase

B. During batch fermentation, in which phase the microbes in the fermenter are adapting to the new environment?

A. **Lag phase** B. Log or exponential phase

C. Stationary phase D. Death phase

C. During the decline phase?

a. Microorganisms try to settle in the new environment

b. Shows exponential growth

c. Death rate is equal to the rate of generation of new cell

d. **Number of live cells decreases**

D. Which of the following is used to grow bacterial cultures continuously?

1. **Chemostat**
2. Coulter Counter
3. Haemostat
4. Petroff-Hausser chamber

3. Microbial Culture Media

A microbial culture medium is a mixture of substances that promotes and supports the growth and differentiation of microorganisms. Culture media contain nutrients, energy sources, growth-promoting factors, minerals, metals, buffer salts, and gelling agents (for solid media). The sophisticated formulations of our culture media ensure precise, reproducible, and repeatable microbiological test result.

Defined media, also known as synthetic media, have a known quantitative and qualitative chemical composition. Every component and its amount in the medium are specified, making it reproducible and consistent. These media are used when studying the minimal nutritional requirements of an organism or for physiological studies, as they allow precise control over the growth conditions.

Complex media are formulated from natural products such as yeast extracts, meat peptones, or beef heart infusion, where the exact chemical composition is not completely known. These media contain a wide range of nutrients. They are used to grow a broad spectrum of microorganisms that may have complex nutritional requirements and are not well-suited to defined media.

1. **Which of the following is a characteristic of beef extract?**

a) Product resulting from the digestion of proteinaceous materials

b) **Aqueous extract of lean beef tissue**

c) Aqueous extract of yeast cells

d) Complex carbohydrate obtained from certain marine algae

2. **Which of the following is used as a solidifying agent for the media?**

a) Beef extract b) Peptone c) **Agar** d) Yeast extract

3. **A culture medium the exact composition of which is not known was called as**

a. Simple b. **Complex** c. Defined d. Natural

4. **When one wants to culture microbes on a large scale for production of useful metabolites, one may not use**

1. sources of nutrients, which are economical and available readily
2. nutrient sources or raw materials that need minimal pretreatment
3. Sources which are available throughout the year
4. **That yields minimum product or biomass per gram of the substrate use**

4. <u>Fermenter</u>

A bioreactor is a type of fermentation vessel that is used for the production of various chemicals and biological reactions.

It is a closed container with adequate arrangement for aeration, agitation, temperature and pH control, and drain or overflow vent to remove the waste biomass of cultured microorganisms along with their products.

A bioreactor should provide for the following:

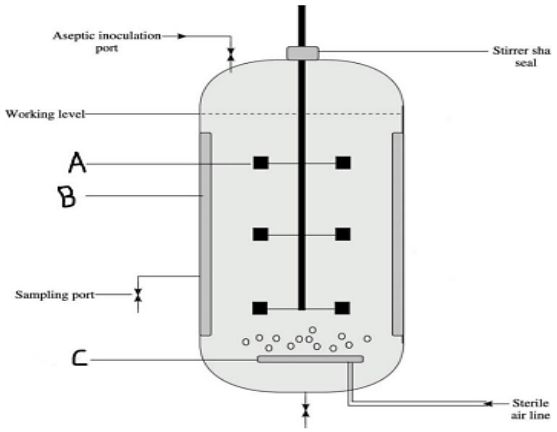
1. Agitation (for mixing of cells and medium),
2. Aeration (aerobic fermenters); for O_2 supply,
3. Regulation of factors like temperature, pH, pressure, aeration, nutrient feeding, and liquid levelled.
4. Sterilization and maintenance of sterility, and
5. Withdrawal of cells/medium

The bioreactor is the heart of any biochemical process as it provides an environment for microorganisms to obtain optimal growth and produce metabolites for the biotransformation and bioconversion of substrates into desirable products.

The reactors can be engineered or manufactured based on the growth requirements of the organisms used.

Reactors are machines that can be made to transform biological-based materials into desirable products. They can be used for the production of various enzymes and other bio-catalysis processes.

1. Identify the parts of fermenter which are labelled as A,B and C



A. Sparger, Baffle , pH Controller
B. Baffle , pH Controller, Sparger
C. Baffle ,Impeller, Sparger
D. Impeller, Baffle, Sparger

2. **The small-scale (seed fermenter) bioreactors have volume of _____**

a) 5-10 litres b) 10-20 litres c) 1-10 litres d) **1-20 litres**

3. **The Batch culture is a/an _____ culture system.**

a) Open b) **Closed** c) Isolated d) Semi-close

4. **Which among them is Not true for batch culture?**

a. The microbial culture goes through a series of growth phases, including lag, exponential, stationary, and death.

b. Nutrients are added continuously throughout the process to maintain growth.

c. The risk of contamination increases over time as the fermentation progresses.

d. The process is typically used for the production of pharmaceuticals where precise control over growth conditions is required.

5. <u>Strain improvement</u>

Strain improvement is an advanced biotechnological strategy where various cellular pathways are modified by recombinant DNA technology to improve the yield of metabolic products that are beneficial to humanity. Strain improvements are directed toward improving product quality and yield by enhancing substrate utilization, regulating enzyme activity, resistance to phage infection, etc.

One of the classical examples of strain improvement using this methodology is the production of antibiotic penicillin. Several successive mutations were necessary to develop a strain of *Penicillium chrysogenum* capable of producing nearly 100 times the concentration of penicillin produced by the original strain *(Penicillium notatum)*, thus making production of penicillin commercially feasible.

The primary genetic routes to strain improvement include (1) mutagenesis for the creation of genetic variants, (2) screening to select improved strains, (3) identification of improved strains, and (4) mass culture optimization of operational and cellular responses and downstream processing.

1. State the principle used in mutant selection. Also give an example of a mutagen.

Ans: The strain is exposed to chemical (e.g. nitrosoguanidine or NTG) or physical (e.g. UV rays) mutagens and the mutants having improved characteristics are selected. e.g. nitrosoguanidine or NTG, UV rays.

2. "A transgene may be added, which encodes an enzyme to modify a metabolite produced by the organism to yield a new product of interest" will this be considered as strain improvement? Justify giving two examples.

Ans: Yes this can be considered as strain improvement. Here the transgene/ foreign gene is introduced through RDT which is a method of genetic engineering that results in improved characters.

3. "When a eukaryotic gene is introduced into a host bacterium, it may not always be expressed" Suggest any reason and measure to overcome this.

Ans. When a eukaryotic gene (e.g., plant, animal, human) is expressed in a prokaryotic (bacterial) host, there are additional problems to be tackled. The non-coding region of the eukaryotic gene must be excised. This requires use of reverse transcription of mRNA into cDNA. Because the bacterial cell does not have the post transcriptional modification.

4. How to check the expression of your foreign gene till they are required?

Ans: The foreign gene may be put under the control of a regulatory switch such that production of recombinant protein does not occur until required

Chapter 2: Plant Cell Culture and Applications

SUMMARY: IMPORTANT POINTS CONCEPTS & DEFINITIONS

1. The unique and the most characteristic property of the plant cell culture is the totipotency.

2. Cell Totipotency is defined as the ability of a plant cell to regenerate into whole plants. During the period 1902 -1930 attempts were made to culture the isolated plant organ such as roots and shoots apices. Next (1940-1970) attempts were made to culture plant tissues, embryos, anthers, pollen, cells and protoplasts, and to regenerate complete plants in suitable nutrient media from cultured tissues and cells.

3. The whole plant can be regenerated virtually from any plant part (referred to as explants or cells. The basic technique of plant tissue culture involves the following steps:

a) Selection of explants such as shoot tips.

b) Surface sterilization of the explants by disinfectants (example sodium hypochlorite) and then washing the explants with sterile distilled water.

c) Inoculation (transfer) of the explants into the suitable nutrient medium (which is sterilized by autoclaving or filter-sterilized to avoid microbial contamination) in culture vessels under sterile conditions (i.e. in laminar flow cabinet).

d) Growing the cultures in the growth chamber or plant tissue culture room, having appropriate physical conditions- (i.e. artificial light (16 h photoperiod), temperature (-26° C) and relative humidity (50-60%).

e) Regeneration of plants from cultured plant tissues.

f) Transfer of plants to the greenhouse or field conditions following the (hardening) of the regenerated plants.

4. The major constituents of a culture medium includes (a) inorganic nutrients,(b) Organic nutrients, (c) Growth hormones for example auxins (cytokinins and gibberellins),(d) Vitamins (for example nicotinic acids, thiamine, pyridoxine) and agar

5. Among the inorganic nutrients, besides C (carbon), H (hydrogen), O (oxygen). Additional 12 elements (N. P. S, Ca,K, Mg, Fe. Mn, Cu, Zn, B, Mb), which are essential for plant growth are included in culture media.

6. Plant hormones play an important role in growth and differentiation of cultured cells in tissues.

7. An optimum pH usually 5 - 7 is maintained.

8. The choice of media varies from plant species to species. The most important media is MS media which was developed by Murashige and Skoog in 1962. MS deals with culture of the isolated organs (like roots) under laboratory condition (in vitro), and different names are given depending upon the organ utilized for the culture.

10. Explant culture. The culture of plant parts (explants) is known as Explant Culture. Explant cultures are generally used to induce callus or plant regeneration. Callus refers to an unorganized mass of cells, which are generally parenchymatous. Callus cultures are used for:

1. Plant regeneration.

2. Preparation of single cell suspensions and protoplast and genetic transformation.

11. Pieces of undifferentiated calli are transferred to medium, which is continuously agitated to obtain a suspension culture. Suspension culture with single cells can also be obtained from intact plant organs either mechanical (grinding the tissue followed by cleaning, filtration and centrifugation) or enzymatic method.

12. Suspension culture can be maintained by any of the two forms-Batch cultures and Continuous cultures.

a) Batch cultures are initiated as single cells in 100-250 ml flasks and are propagated by transferring regularly small aliquots of suspension to a fresh media.

b) Continuous cultures, are maintained in a steady state for long period by draining out the used and adding fresh medium back to suspension culture or the addition of medium is accompanied by the harvest of an equal volume of suspension culture (these are called *open continuous culture").

13. Organogenesis means formation of organs from cultured explants. In Somatic embryogenesis, the totipotent cells may undergo an embryogenic pathway to form somatic embryos which can be grown to regenerate into complete plants.

14. Somatic Embryogenesis is influenced by plant extract, growth regulators and by the physiological state of calli.

15. The tissue culture methods of plant propagation, known as micro propagation utilizes the culture of apical shoots, axillary buds and meristems on suitable nutrient medium.

Significance of this Method

1. Rapid multiplication of superior clones and maintenance of uniformity.

2. Multiplication of disease free plants.

3. Multiplication of sexually derived sterile hybrids.

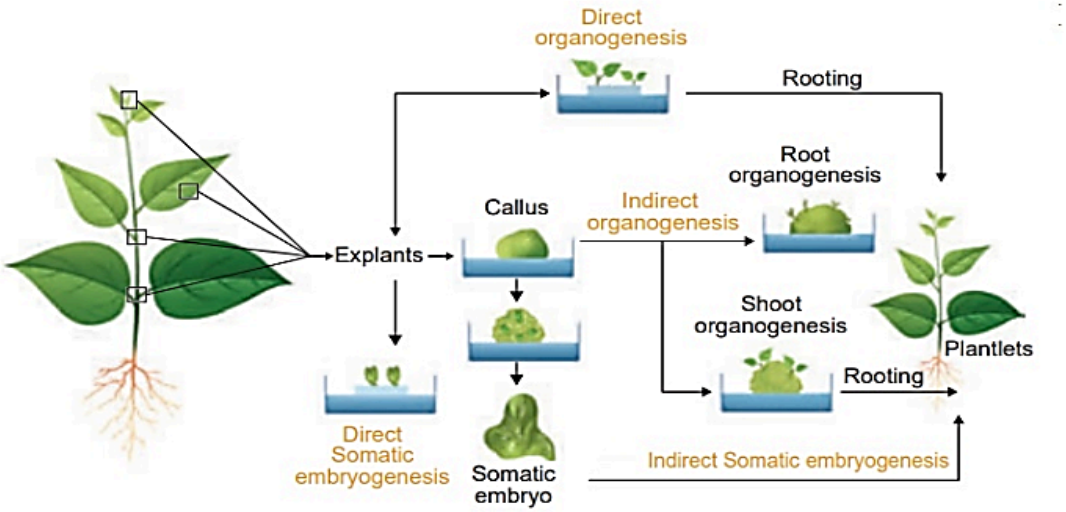


Figure 5.10: Plant Regeneration Pathway

4. The micro propagation is rapid and has been adopted for commercialization of important plants such as banana, apple, pears, strawberry, cardamom, many ornamental (example Orchids) and other plants. In certain crops, and particularly in ornamental plants, it has become possible to produce virus free plants through tissue culture at the commercial level. In the conventional plant tissue culture for clonal propagation, storage and transportation of propagules for transplantation is a major problem.

16. The artificial seeds (also called as synthetic seeds or somatic seeds) can be utilized for the rapid and mass propagation of elite plant species as well as hybrid varieties.

17. The term "embryo rescue" is used for embryo culture, ovule culture and ovary culture, since in each case the objective is to rescue the embryo. During distant hybridization, often the embryo aborts at an early stage of development, so that no mature seed can be obtained.

18. Also it is very difficult to produce hybrids in case of interspecific and intergenetic crosses because of abnormal development of the endosperm which causes premature death of the hybrid embryo and leads to formation of sterile seeds.

19. The technique of haploid production through anther and pollen culture as well as ovary culture is of immense use in plant breeding employed to improve crop plants.

20. Triploid plants raised by endosperm culture show seed sterility or seedlessness which is desirable in crops like citrus, apple and pear.

21. Protoplast Fusion or Somatic Hybridization is one of the most important uses of protoplast culture. This is particularly significant for hybridization between species or genera, which cannot be made to cross by conventional method of sexual hybridization. Somatic hybridization is generally used for fusion of protoplasts either from two different species (interspecific fusion) or from two diverse sources belonging to the same species.

22. In 1972, the first interspecific somatic hybrid between *Nicotiana glauca* and *N. langsdorffii* was produced by Carlson and his associates. Later, Melchers and his team in 1978 developed the first inter-genetic somatic hybrids between *Solanum tuberosum* (potato) and *Lycopersicon esculentum* (tomato) and the hybrids are known as 'Pomatoes or Topatoes.

23. Plants produce thousands of sophisticated chemical molecules. These include chemicals that are required for the plant's basic metabolic processes such as sugars, lipids, amino acids, nucleic acids and secondary metabolites such as alkaloids, resins, tannins, latex, etc. However, many of the secondary products, especially various alkaloids, are of immense use in medicine.

24. Such compounds are produced in plants in small amounts and therefore, they are quite expensive. Certain plant products such as Shikonin are being produced commercially in large scale using bioreactors.

Table 1. Few examples of industrially important plant secondary metabolites produced through cell and tissue cultures

Product	Plant source	Uses
Artemisin	*Artemisia* spp.	Antimalarial
Azadirachtin	*Azadirachta indica* (Neem)	Insecticidal
Berberine	*Coptis japonica*	Antibacterial, Antiinflammatory
Capsaicin	*Capsicum annuum* (chilli)	Reumatic pain treatment
Codeine	*Papaver* spp.	Analgesic
Digoxin	*Digitalis lanata*	Cardiac tonic
Diosgenin	*Dioscorea deltoidea*	Antifertility
Scopolamine	*Datura stramonium*	Antihypertensive
Quinine	*Cinchona officinalis*	Antimalarial
Shikonin	*Lithospermum erythrorhizon*	Antimicrobial; Red pigment used in lipstics & dye for silk
Taxol	*Taxus* spp.	Anticarcinogenic
Vincristine	*Cathranthus roseus*	Anticarcinogenic

25. In vivo gene banks have been made to preserve the genetic resources by conventional methods e.g. seeds, vegetative propagules, etc. In vitro gene banks have been made to preserve the genetic resources by non-conventional methods, i.e. cell and tissue culture methods.

26. Freezing Storage or Cryopreservation: This utilizes the long term preservation of cell and tissues at ultra-low temperature (-196°C i.e. in liquid nitrogen) for indefinite time by using cryoprotectants (example, dimethyl sulfoxide, glycerol, proline and mannitol).

27. In the direct gene transfer methods, the foreign gene of interest is delivered into the host plant cell without the help of a vector.

Transfection: Direct DNA uptake by protoplasts can be done by chemicals like polyethylene glycol (PEG) and dextran sulphate. The technique is so efficient that virtually every protoplast system has proven transformable.

Microinjection: Here the DNA is directly injected into plant protoplasts or cells (specifically into the nucleus using fine tipped (0.5-10 micrometers diameter) glass needle or micro pipette.

Electroporation: This method is based on the use of short electrical impulses of high field strength.

Biolistic: In recent years, it has been shown that the DNA delivery to plant cells is also possible, when heavy micro particles (tungsten or gold) coated with the DNA of interest are bombarded with initial Velocity (1400 ft per sec.).

28. The biotic stress that plants encounter is viral, bacterial, fungal pathogens, nematodes, insect pests and weeds. Weed (plants growing where they are not wanted) decreases crop yields and quality primarily by competing with crop plants for light, water and nutrients.

29. The most commonly employed approach is the over-production of herbicide target enzymes (usually in the chloroplast) in the plant, so that it becomes insensitive to the herbicide. All crop plants are affected by a variety of insects, mites and nematodes that significantly reduce their yield and quality.

30. The transgenic technology provides an alternative and innovative method to Improve pest control management which is eco-friendly, effective, sustainable and beneficial in terms of yield. Transgenic crops (e.g. cotton, rice, maize potato. tomato, brinjal, cauliflower, cabbage etc.) with Bt genes have been developed.

Such transgenic varieties proved effective in controlling the insect pests and it has been claimed worldwide that it has led to significant increase in yield along with dramatic reduction in pesticide use. In this a single gene imparting the virus resistance is transferred and transgenic plants are produced.

31. Abiotic stress response reactions involve the production of stress related osmolytes like sugars, eg: trehalose and fructans, sugar alcohols, eg:mannitol, amino acids ex:proline, glycine, betaine and certain Proteins eg. Antifreeze proteins.

32. The gas hormone, ethylene, is involved in the regulation of fruit ripening. Therefore, ripening can be slowed down by blocking or reducing ethylene production.

33. Male sterile plants are very important to prevent unnecessary pollination and to eliminate the process of emasculation during the production of hybrid plants. These are created by introducing a gene coding for an enzyme (barnase, which is an RNA hydrolyzing enzyme) that inhibits pollen formation.

34. In 1990, C. Mariani and others from Belgium successfully used a gene construct having another specific promoter (from TA 29 gene of tobacco) and bacterial coding sequence for a ribonuclease (Barnase gene from *Bacillus amyloliquefaciens)* for production of transgenic plants.

Utilizing this male sterility barnase gene construct (TA-29-RNase), it was possible to introduce male sterility in other crops also, for example tobacco, lettuce, cauliflower, cotton, tomato, corn etc.

35. An additional major goal of the biotechnology industry is also the use of transgenic plants and factories for manufacturing specialty chemicals and pharmaceuticals.

36. Transgenic crops with improved nutritional quality have already been produced by introducing genes involved in the metabolism of vitamins, minerals and amino acids. For example, Vitamins-A deficiency can lead to night blindness and skin disorders, among others. Prof. Ingo Potrykus and Dr. Peter Beyer developed genetically engineered rice (popularly known as 'Golden Rice') which is enriched in pro-vitamin A by introducing three genes involved in the biosynthetic pathway for carotenoid, the precursor for Vitamin-A.

37. The nutrition quality of cereals and legumes are limited because of deficiency of the essential amino acids, i.e. lysine in cereals and methionine and tryptophan impulses.

38. Transgenic plants can also be used to express antigens which can be used as Edible vaccines. The procedure involves the isolation of genes encoding antigenic proteins from the pathogens expressed in plants. The edible vaccines that are produced like this have the following advantages like (a) Alleviation of storage problems, (b) Easy delivery systems by feeding (c) Less costs as compared to the recombinant vaccine produced by bacterial fermentation.

The major concerns about GM crops and GM foods are -

1. The safety of GM food for human and animal consumption (for example, GM food may cause allergenicity)

2. The effect of GM crops on biodiversity and environment.

3. The effect of GM crops on non-target and beneficial insects/microbes.

4. Transgenes may escape through pollen to related plant species (gene pollution) and may lead to the development of super weeds.

5. The GM crops may change the fundamental vegetable nature of plants as the genes from animals (e.g. fish or mouse) are being introduced into crop plants.

6. The antibiotic resistance marker genes used to produce transgenic crops may horizontally transfer into microbes and thus exacerbate the problem of antibiotic resistance in human and animal pathogens.

7. The GM crops may lead to the change in the evolutionary pattern.

However, with the increase in the awareness about the benefits and hazards of plant genetic engineering and gene technology people by and large are now realizing the immense potential and the benefits of GM crops. We should make sure that people adhere to the specific standard regulatory policies formulated and adopted by various nations regarding commercial use of GM foods.

MULTIPLE CHOICE QUESTIONS

1. Who is known as the father of tissue culture?

(a) Bonner (b) Laibach (c) **Haberlandt** (d) Gautheret

2. The production of secondary metabolites requires the use of _____.

(a) Meristem (b) Protoplast (c) Axillary buds (d) **Cell suspension**

3. The pair of hormones required for a callus to differentiate are_____.

(a) Ethylene and Auxin (b) **Auxin and Cytokinin**

(c) Auxin and Abscisic acid (d) Cytokinin and gibberellin

4. What is Dimethyl sulfoxide used for?

(a) A gelling agent (b) **Cryoprotectant** (c) Chelating agent (d) An Alkylating agent

5. The formation of embryoids from the pollen grains in the tissue culture medium is due to _____.

(a) Organogenesis (b) Test tube culture (c) Double fertilization **(d) Cellular totipotency**

6. Synthetic seeds are produced by the encapsulation of somatic embryos with_____.

(a) Sodium acetate (b) Sodium nitrate (c) Sodium chloride (d) **Sodium alginate**

7. Totipotency refers to _____.

(a) Development of fruits from flowers in a culture

(b) **Development of an organ from a cell in a culture medium**

(c) Flowering in a culture medium

(d) All of the above

8. Which of the following is the main application of embryo culture?

(a) Clonal propagation (b) Production of embryoids

(c) Induction of somaclonal variations (d) **Overcoming hybridisation barriers**

9. In tissue culture of parenchyma, mitosis is accelerated in the presence of _____.

(a) Auxin (b) Cytokinin (c) Gibberellin (d) **Both auxin and cytokinin**

10. In which of the following conditions do the somaclonal variations appear?

(a) **Plants raised in tissue culture**

(b) Plants exposed to gamma rays

(c) Plants growing in polluted soil or water

(d) Plants transferred by a recombinant DNA technology.

11. Haploid plants can be obtained from_____.

(a) **Anther culture** (b) Bud culture (c) Leaf culture (d) Root culture

12. In-plant tissue culture, the callus tissues are generated into a complete plantlet by altering the concentration_____.

(a) Sugars (b) **Hormones** (c) Amino Acids (d) Vitamins and minerals

13. Which of the following is cultured to obtain haploid plants?

(a) Embryo (b) Nucleus (c) Apical bud (d) **Entire anther**

14. Which of the following vectors is used in crop improvement and crop management?

(a) **Ti plasmid** (b) BAC (c) Cosmid (d) Phasmid

15. Which of the following growth hormones produces apical dominance?

(a) Ethylene (b) Cytokinin (c) **Auxin** (d) Gibberellin

16. Cybrids are produced by?

(a) **The nucleus of one species but cytoplasm from both the parent species**

(b) The fusion of two same nuclei from the same species

(c) The fusion of two different nuclei from different species

(d) None of the above

17. Which of the following mediums is composed of chemically defined compounds?

(a) Natural media (b) Artificial media **(c) Synthetic media** (d) None of the above

18. Which of the following chemicals are most widely used for protoplast fusion?

(a) Mannitol **(b) Polyethylene glycol** (c) Sorbitol (d) Mannol

19. Which of the following plant cells shows totipotency?

(a) Cork cells **(b) Meristem** (c) Sieve tube (d) Xylem vessels

20. What is Callus?

(a) Tissues that grow to form an embryoid

(b) An unorganized actively dividing the mass of cells maintained in a culture

(c) An insoluble carbohydrate

(d) A tissue that grows from an embryo

21. What are somaclones?
a) Plants chemically identical to the original plant
b) Plants morphologically identical to the original plant
c) Plants anatomically identical to the original plant
d) Plants genetically identical to the original plant

22. What is protoplast?
a) Cell wall + Plasma membrane **b) Plant cell – cell wall**
c) Cytoplasm + cell wall d) Plasma membrane – cytoplasm

23. The typical plant tissue culture nutrient medium consists of :

a) Inorganic salts,a carbon source (usually sucrose), vitamins, amino acids , growth regulators and compounds like casein hydrolysate, coconut milk, malt extract, yeast extract, tomato juice.

b) Inorganic salts, vitamins, amino acids, growth regulators and compounds like casein hydrolysate, coconut milk, malt extract, yeast extract, tomato juice.

c) Inorganic salts,a carbon source (usually sucrose), vitamins, growth regulators and compounds like casein hydrolysate, coconut milk, malt extract, yeast extract, tomato juice.

d) Inorganic salts,a carbon source (usually sucrose), amino acids , growth regulators and compounds like casein hydrolysate, coconut milk, malt extract, yeast extract, tomato juice.

24. The explants can be any part of the plant like

a. piece of stem b. leaf c. hypocotyls d. cotyledon

1. only a true
2. only a and b true
3. only a,b,c true
4. **a, b, c, d are true**

25. Isolated and purified organelles-chloroplasts or mitochondria from one species can be fused with the recipient protoplasts from a different plant species is called

a) Organelle transfer **c) Both a and b**

b) Organelle uptake d) None of these

26. I. Preservation of the genetic resources by seeds, vegetative propagules, etc. and it is known as (***in vivo* gene banks**)

II. Preservation of the genetic resources through Cell and tissue culture methods ***in vitro* gene banks**

1. only I true
2. only II true
3. neither I nor II is true
4. **both I and II are true**

27. For delayed ripening the strategy used involve

a. by blocking or reducing ethylene production by RDT

b. introducing ethylene forming gene(s) in a way that will suppress its own expression in the crop plant

1. only a
3. only b
2. **both a and b**
4. another strategy is to be use

28. The production of novel hybrids through protoplast fusion should focus on ……..

a) Agriculturally important traits

b) Somatic hybrids integrated into a conventional breeding programme

c) Extension of protoplast regeneration to a wider range of crop species

d) **All the above**

29. The relatively high level of auxin to cytokinin favoured ……. during organogenesis.

a) **Root formation**
b) Shoot formation
c) Both root and shoot formation
d) All of the above

30. Genesis of an entire plant from cultured explants directly or via callus indirectly is called

a). Differentiation
b). De-differentiation
c). Re-differentiation
d). **Regeneration**

ASSERTION REASON QUESTIONS

Directions: In the following questions, a statement of assertion is followed by a statement of reason. Mark the correct choice 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

1. **Assertion**: In plant tissue culture, somatic embryos can be induced from any plant cell.
Reason: Any viable plant cell can differentiate into somatic embryo.

Ans : A

2. **Assertion**: The process of plant tissue culture can be used for faster vegetative propagation of new crop varieties.

Reason: Cells in a callus are totipotent in nature.

An: A

3. **Assertion:** Transgenic plant production is an application of plant tissue culture.

Reason: An organism that contains and expresses a transgene is called a transgenic organism.

Ans: B

4. **Assertion:** 'Cry' proteins are named so because they are crystal proteins.

Reason: In the acidic environment of insect midgut 'Cry' proteins are solubilized and then release toxic core fragments after photolytic action.

Ans: A

5. **Assertion:** The process of plant tissue culture can be used for faster vegetative propagation of new crop varieties.

Reason: Cells in a callus are totipotent in nature.

Ans: A

6. **ASSERTION**: Virus free plants are regenerated through apical meristem culture.

REASON: Apical meristem cells are devoid of viral infection.

Ans: a

7. **ASSERTION**: The Somatic cell hybridization offers an excellent alternative for obtaining distant hybrids.

REASON: Somatic hybrids are created by fusing intact cells of distantly related plants.

Ans: a

8. **ASSERTION**: Auxins are added to the culture medium if callus induction is desired.

REASON: Nature and quantity of auxin added depends on nature and source of explant.

Ans: b

9. **ASSERTION**: Male sterile plants can be created by introducing plants with barnase gene from *Bacillus amyloquifaciens*.

REASON: Barnase gene under control of TA29 promoter produces RNA hydrolyzing enzyme in tapetal cells which inhibits pollen formation.

Ans: a

10. **ASSERTION**: There are no constraints associated with public acceptance of transgenic crops.

REASON: Transgenic crops are completely not safe for all forms of life and can cause any threat to evolution and biodiversity.

Ans: d

SHORT ANSWER QUESTIONS

1. **What is cell totipotency?**

Ans: It is defined as the ability of a plant cell to regenerate into a whole plant.

2. **Which property of the plant cell is exploited to culture plant cells?**

Ans: The property of cell toti-potency is exploited to culture the plant cells. Cell toti-potency is the ability of a plant cell to regenerate into a whole Plant.

3. **What is explant?**

Ans: The excised plant part from which the whole plant can be regenerated is called explant.

4. **Which is the most commonly used culture media?**

Ans: The most extensively used nutrient medium is MS medium which was developed by Murashige and Skoog in 1962.

5. **Which protein is required in culture medium for callus induction?**

Ans: Auxins.

6. **Which chemical compound is used as disinfectant for surface sterilization of the explants.**

Ans: Sodium hypochlorite.

7. **Name the plants for which micropropagation technique is used commercially.**

Ans: The technique of micropropagation has been commercially used for plants like banana, apple, etc.

8. **Give two examples of plants grown using callus culture.**

Ans: Brinjal-where leaf explant is used to grow multiple shoots. Tobacco-leaf mesophyll cells used to grow in culture.

9. **What are Cybrids?**

Ans: Cybrids are cytoplasmic hybrids created by the fusion of enucleated and nucleated protoplasts.

10. **What is the name of the first inter-genetic somatic hybrids between potato and tomato?**

Ans: The first intergenetic somatic hybrids between potato and tomato are known as " Pomatoes" "Topatoes"

11. **Name of few compounds used as cryoprotectants?**

Ans: Dimethyl sulfoxide, glycerol, proline and mannitol are examples of Cryoprotectants.

12. **Name the bacteria known as natural genetic engineer of plants.**

Ans: *Agrobacterium tumefaciens* is the bacteria which are known as natural genetic engineer of plants.

13. **Name the protective chemical in which the embryos are encapsulated in artificial seeds.**

Ans: The protective chemical is calcium alginate.

14. **Name the red algae from which the gelling agent agar is obtained.**

Ans: The gelling agent agar is obtained from a red algae *Gelidium amansi.*

SHORT ANSWER QUESTIONS- 2 MARKS

15. **Enumerate the different steps in micropropagation methods.**

Ans: There are four different steps in the Micropropagation method. Steps are

i. Initiation of culture. ii. Shoot formation. iii. Rooting of shoots iv. Transplantation

16. **Write briefly the benefits of biodegradable plastics that are produced from GM plants.**

Ans: Biodegradable plastic which is produced from GM plant are: -

i.Economic ii.Conserve nature iii.Eco Friendly

17. **Explain how embryo rescue can be used to produce novel hybrids.**

Ans: It is very difficult to produce hybrids in case of interspecific & intergeneric crosses due to abnormal development of endosperm which causes premature death of hybrid embryo and leads to formation of sterile seed.

ii. Novel hybrid is produced by exercising the embryo from sterile hybrid seeds at an appropriate time along with culturing it with a suitable Nutrient medium. This is known as embryo rescue.

18. **Explain why Bt cotton flowers undergo pollination by butterflies and bees in spite of being insect pest resistant**.

Ans: Because butterflies and bees are not a pest and therefore, they do not cause any disease or harm to the plants. They simply come in contact with the flowers for nectar and do not eat any part of the flower. Hence, they only aid pollination and do not die due to toxins in Bt Cotton.

19. **What are the two methods used for isolation of single plant cells?**

Ans: Both mechanical and enzymatic methods can be used for isolation of plant cells. Mechanical methods involve grinding of the tissue to a fine suspension in a buffered medium followed by filtration/ centrifugation to get rid of cell debris. The enzymatic method is based on the usage of enzymes (pectinase/ macerozyme) which dissolve the middle lamella between the cells i.e. the intercellular cement to release single cells.

20. **What are the uses of cell suspension cultures?**

Ans: The cell suspension cultures are used for:

a. Induction of somatic embryos/shoots

b. In vitro mutagenesis and mutant selection.

c. Genetic transformation.

d. Production of secondary metabolites.

21. **What is meant by 'Golden Rice'? In what way is it different from the Normal rice?**

Ans: Prof. Ingo. Potrykus and Dr. Peter Beyer developed a genetically engineered Rice known as Golden Rice which is enriched in pro-vitamin A by Introducing three genes involved in the biosynthetic pathway for carotenoid, the precursor for vitamin A. The seeds of Gold Rice are yellow in colour because of provitamin A which is produced in the entire grain.

SHORT ANSWER QUESTIONS- 3 MARKS

22. **What are genetic engineering strategies to create the following traits in transgenic crops?**

a. Herbicide tolerance

b. Abiotic stress tolerance

c. Insect resistance.

Ans: Biotechnology strategies can be used to overcome limitations posed by application of chemical pesticides.

a. Weeds decrease the crop yields and quality by competing with crop plants for light, water and nutrients. So far, the control of weeds is achieved by engineering the crop by over production of herbicide target enzymes. Plant becomes insensitive to the herbicide.

b. Transgenic plants over express the genes for sugar, alcohol, amino acids etc. which increases plant tolerance to environmental stress.

c. Genetic modification of crops provides plants with genes which are pest resistance, Eg-crygene.

23. **What was the name of the first gene available for genetic engineering of crop plants for pest resistance?**

Ans: CryIAC gene

24. **What are the natural secondary metabolites produced from plants?**

Ans: Plants produce thousands of sophisticated chemical molecules. These include the chemicals that are required for the plant's basic metabolic processes such as alkaloids, resins, tannins, latex etc. Shikonin is obtained from *Lithospermum erythorizon* and is used as antimicrobial, red pigment used in lipsticks and dye for silk. Quinine obtained from *Cinchona officinalis* is used as an antimalarial drug.

26. **Write two genetic engineering approaches which have been used to improve the seed protein quality.**

Ans: We can improve the quality of protein in the seed by -

Introducing the amino acids (containing sulphur rich amino acid) into a pea plant for removing the deficiency of methionine and cysteine.

ii. Modification of endogenous genes to increase lysine in seed proteins of cereals.

27. **What are edible vaccines and what are their uses?**

Ans: Edible vaccines are the antigens expressed in some crop plants which are eaten for vaccination/immunization. These are low cost vaccines Compared to recombinant vaccines. The genes encoding antigen are isolated from pathogens and expressed in such transgenic plants. Uses:

i. Vaccinating Hepatitis and cholera plants by feeding Banana and Tomato.

ii. Feeding animals sugar beets to vaccinate them against foot and mouth disease.

28. **How can the nutritional deficiencies of seeds be overcome? Give Examples.**

Ans: This is achieved by using engineer genes which encodes storage proteins in a seed with more nutritionally desirable amino acid. This is achieved by inserting additional amino acid or substituting undesirable amino acid with new one. Eg. Zein storage.

29. **What is the special precaution taken while culturing plant cells in plant bioreactors?**

Ans: Plant cells can be cultured in specially designed "Plant bio reactors" which essentially do not have a stirrer (as plant cells are shear sensitive). In place of stirrer, gas is gently bubbled which provides stirring as well as meet the demand of a higher oxygen supply.

30. **What is protoplast culture? Write its significance.**

Ans: Protoplasts are plant cells without a cell wall and can be isolated by enzymatic methods (cellulases, hemicellulases and pectinases) from leaf, seedling, calli, pollen grain, embryo sacs etc. As the protoplast lack cell wall, they can be utilized for many purposes such as:

a) Various biochemical and metabolic studies.

b) Fusion of two somatic cells to create somatic hybrids.

c) Fusion of enucleated and nucleated protoplasts to create cybrids.

d) Genetic manipulation.

31. **Write down the composition of nutrient media which is used for plant Tissue culture.**

Ans: The most extensively used nutrient medium is MS medium, developed by Murshige and Skoog. It consists of the following-

- Inorganic salts (both macro and micro-elements).
- A carbon source (usually sucrose)
- Vitamins (e.g. nicotinic acid, thiamine, pyridoxine and Myo-inositol).
- Amino acids (e.g. arginine)
- Growth regulators (e.g. auxins, cytokinins and gibberellins) vi. Some other compounds like casein hydrolysate, coconut milk, Malt extract, yeast extract, tomato juice etc. (Agar- gelling agent agar is added to the liquid medium for its solidification).

32. **How can the ripening of the fruit be delayed? What commercial importance can this serve?**

Ans: The ripening of the fruit is delayed by Introducing ethylene forming gene(s) to suppress its expression in the crop plant. This makes fruit ripen slowly. Due to slow ripening (ethylene gene regulation) the fruits can be exported to longer distances without spoilage as they show longer shelf life.

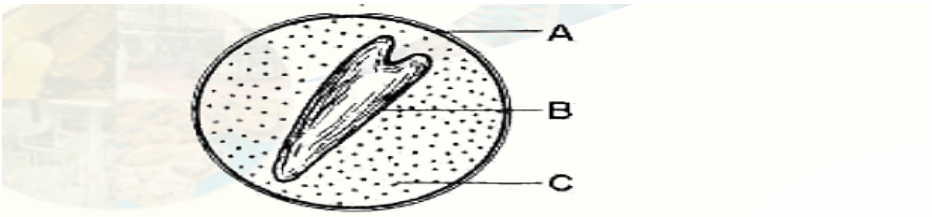
33. **How do the transgenic plants used as factories to produce Biodegradable plastics?**

Ans: Transgenic plants are used as factories because: Genetically engineered *Arabidopsis* plants produce PHB (poly hydroxyl butyrate) globules exclusively in their chloroplast without affecting Plant growth and development from leaves ex. Populus. The large-scale production of PHB is easily achieved by extracting PHB from leaves.

34. **What are artificial seeds?**

Ans: Artificial seeds are the embryos encapsulated in protective chemicals like calcium alginate which prevents them from desiccating. They can be utilized for the rapid and mass propagation of elite plant species as well as hybrid variety.

Encapsulated in protective chemicals prevents them from desiccating. They can be utilized for mass propagation of elite plant species as a hybrid variety.



The diagrammatic representation of an artificial seed. The artificial seed coat (A), somatic embryo at torpedo stage (B) and artificial endosperm (C).

35. **Who first attempted to cultivate the mechanically isolated plant leaf cells on a simple nutritive medium? Mention its contribution.**

Ans: Gottlieb Haberlandt was the first to attempt to culture the mechanical isolated plant leaf cells on a simple nutrient medium.

Contribution: Concept of growth hormones, the use of embryo sac fluids, the cultivation of artificial embryos from somatic cells, etc.

LONG ANSWER QUESTIONS -5 MARKS EACH

36. **Describe Bioethics in plant genetic engineering.**

Ans: The GM crops are fast becoming a part of agriculture throughout the world because of their contribution to increased crop productivity and to global food and fibre security, besides their use in health care and industry However, the constraints associated with public acceptance of transgenic crops continue to be important challenges facing the global community. The following are the major concerns about GM Crops and GM foods.

1. The production of genetically modified plants may cause some allergic reactions in the long run.

2. Transgenic plants may lead to proliferation of new viral, fungal and insect strains.

3. Overuse of Transgenic plants has the ecological risk of developing into weeds.

4. Over growth of transgenic may lead to extinction risk or developing into weeds.

5. Some transgenic plants carry the genes from moves and other animals which is likely to alter the basic nature of plants and may cause serious diseases.

6.The GM crops may lead to the change in the evolutionary pattern.

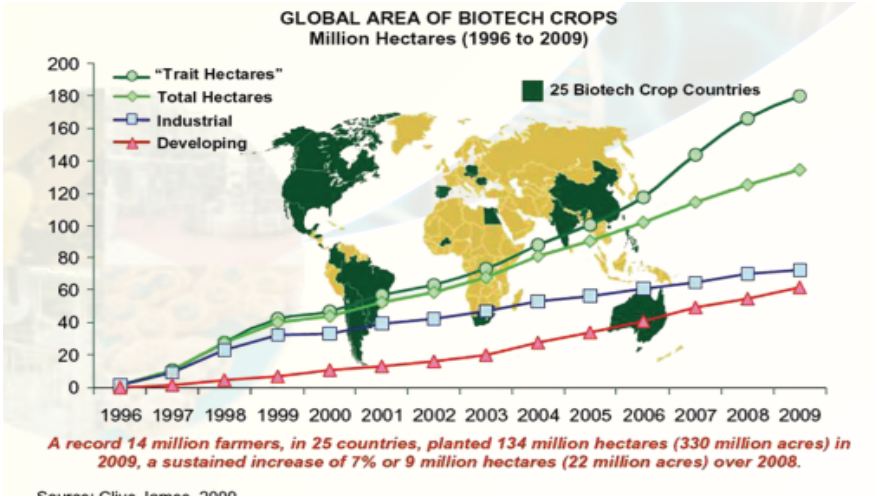
7. The antibiotic resistance marker genes used to produce transgenic crops may horizontally transfer into microbes and thus exacerbate the problem of antibiotic resistance in human and animal pathogens.

Biotechnology is a double-edged sword and depends entirely on the manner of its application. Science & technology are meant for the good of the society and drifting from its traditional goals would be disastrous and lead to unknown hazards.

CASE BASED QUESTIONS

1. The GM crops may lead to the change in the evolutionary pattern.

Unfortunately, the public debate over the benefits and hazards of plant gene technology suffers from an astounding array of misinformation, misunderstanding and manipulation. In fact, with the continuing accumulation of evidence of safety and efficiency, and the complete absence of any evidence of harm to the humans and animals as well as the environment, more and more consumers are becoming comfortable because of the increased awareness about the potential of the plant genetic engineering. Further, the transgenic crops (e.g. cotton, tomato, corn and soybean) which have already entered the marketplace (which carry a label, i.e., GM crop or GM food in several countries were subjected for the extensive field trials for environmental safety related to wild species and competitive performance of transgenic and toxic effects as per the standard regulatory policies before they were approved for commercialization. In fact, in 2009, a record of 14 million farmers from 25 countries cultivated 134 million hectares (330 million acres) with crops that were genetically engineered for herbicide, insect resistance, delayed fruit ripening and improved oil quality. The global area of biotech crops planted from 1996 to 2009 is shown in



1**. What are transgenic crops? Give two examples.**

Ans. Genetically modified crops are known as transgenic crops. Flavr savr tomato and Bt cotton

2. **From the above given data enumerate the growth of biotech crops (trait hectors) from 2006 to 2009**

Ans: 110 million hectares to 180 million hectares.

3. **Why do biotech crops suffer from public debate? give three reasons.**

Ans: Biotech crops suffer from a public debate array of misinformation, misunderstanding and manipulation.

4. **"Even after so many misconceptions, the biotech products are now being globally accepted".justify by giving reason.**

Ans: The continuing accumulation of evidence of safety and efficiency, and the complete absence of any evidence of harm to the humans and animals as well as the environment, more and more consumers are becoming comfortable to accept biotech products. In 2009, a record of 14 million farmers from 25 countries cultivated 134 million hectares (330 million acres) with the crops that were genetically engineered for herbicide, insect resistance, delayed fruit ripening and improved oil quality.

2. Artificial seeds

An artificial seed (also called a synthetic seed or synseed, seed analog, or manufactured seed) includes a range of plant structures, including somatic embryos, buds, shoots, or other meristematic tissues inside a coating, that can be sown in the same way as a conventional seed to produce a new plant. The coating may be water impermeable or water soluble and may also enclose nutrients (artificial endosperm) and other additives deemed necessary (e.g., mycorrhizal fungi, fungicides, and/or bacteriocides).

The artificial seed production technique was first used in clonal propagation to cultivate somatic embryos placed into an artificial endosperm and constrained by an artificial seed coat. Today artificial seeds represent capsules with a gel envelope, which contain not only somatic embryos but also axillary and apical buds or stem and root segments. Explants such as shoot tips, axillary buds and somatic embryos are encapsulated in cryoprotectant material like hydrogel, alginate gel, ethylene glycol, dimethylsulfoxide (DMSO) and others that can be developed into a plant. The coating protects the explants from mechanical damage during handling and allows germination and conversion to occur without inducing undesirable variations. They behave like true seeds and sprout into seedlings under suitable conditions.

1. Synthetic seeds are

A. artificially synthesized seeds

B. somatic embryos encapsulated in suitable matrix

C. seeds of plants modified genetically

D. none of these

2. **Somatic embryos are**

A. identical with zygotic embryos and without seed coats

B. identical with zygotic embryos and with seed coats

C. non-identical with zygotic embryos and without seed coats

D. non-identical with zygotic embryos and with seed coats

3. **Which of the following is not true about synthetic seeds?**

A.Can be stored for a year without the loss of valuables

B.Easy to handle

C.Can be directly sown in the soil like natural seeds

D.**Need hardening in the green house**

4.**The encapsulation of somatic embryos can be carried out by**

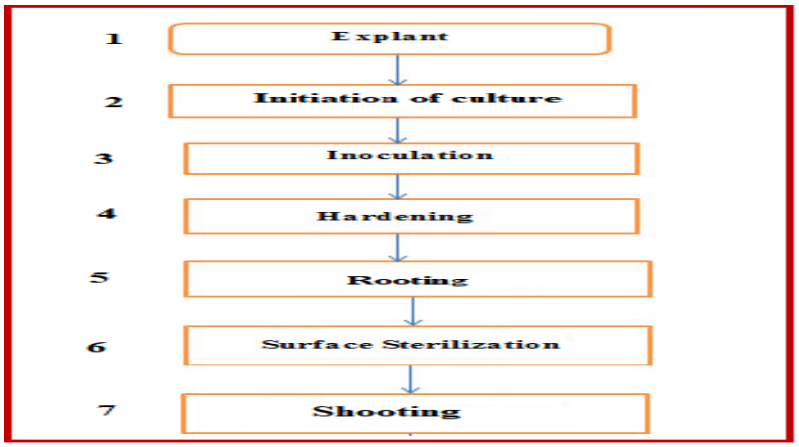
A.automatic encapsulation process B.gel complexation

C.**both (a) and (b)** D.coating proteins

3. Micropropagation

The term “micropropagation” refers to the large-scale multiplication of plants by bud proliferation or bud induction on plant culture media in vitro. It is implicit that when micropropagation is used commercially as an alternative to vegetative propagation, the plants produced should be true-to-type, free of pathogens, and free of microbial contaminants. The latter are environmental organisms that enter the cultures on or in the explant at establishment of the tissue culture, or enter during the serial subculture steps in multiplication. Vegetative multiplication of plants in the field is a slow process that is dependent on plant growth cycles, requires labor for pesticide treatments, irrigation, etc., and is at risk from crop infection or loss due to abiotic and biotic stresses, e.g., drought and disease, respectively.

1. **Arrange the below given flow chart in the correct order**



Ans: 1-6-3-2-7-5-4

2. Do you think micropropagation is a technique that has great significance in the field of agriculture? Justify giving suitable reasons to support your answer.

Ans: Vegetative propagation of plants is of considerable importance in agriculture, horticulture and forestry as it provides the multiplication of uniform material for crop planting (Clones). Traditional methods of vegetative propagation are labour-intensive, low productivity and seasonal. Thus, tissue culture method of plant propagation, known as 'micro propagation' that can overcome the problems mentioned above.

3. Could we sterilize the plant tissue in the autoclave? Why or why not?

Ans. No, delicate cells cannot be done through autoclaving, since the procedure has a high temperature. If we tried to sterilize plant materials using high heat, we would kill the plant.

4. Why is a sterile environment important in tissue culture?

Ans. An environment that has been treated in a way so that it will be free from all bacteria, viruses and fungi.

UNIT- 6

Cell Culture and Genetic Manipulation

Chapter- 3: Animal Cell Culture and Applications

Cell Culture: The process of growing animal (or plant) cells outside the organism under controlled lab conditions. Grown in vitro (Latin: *within glass*), as opposed to in vivo (*within the living*).

Clone: A homogeneous population of cells derived from a **single parental cell**.

- All cells in a clone are **genetically identical**.

HeLa Cells: First human cell line developed by **George Gey** in the **1950s** from **cervical cancer**.

Slow division rate: Animal cells divide approximately every **18–24 hours**. This slow rate makes them **vulnerable to contamination**, especially from faster-growing microorganisms like bacteria.

Properties of Animal cells in culture:

- **Limited Cell Division:** Normal animal cells in culture have a finite number of divisions, mirroring the limited growth of organisms to adulthood.
- **Contact Inhibition:** Normal cells stop dividing when they reach confluence in culture, similar to how tissues and organs in the body cease growing after reaching a certain size. This is termed "contact inhibition".
- **Culture Environment Effects:** The in vitro environment differs from the in vivo environment, impacting cell adherence, shape, and proliferation rate.
- **Cancer Cell Differences:** Cancer cells in culture lack contact inhibition, leading to piling and a more rounded shape, contrasting with normal cells.
- **Colony Formation Assay:** Oncologists use differences in growth patterns, like contact inhibition, in assays to distinguish cancerous from non-cancerous tumours.

Cell Isolation for Culture

Cells are isolated from **tissues** (e.g., liver, kidney) using:

- **Mechanical methods**
- **Enzymatic digestion**: Using enzymes like:**Trypsin, Collagenase.** These enzymes **break the intercellular matrix** to free cells.

Primary Cell Culture: Initial culture from freshly isolated cells, Characteristics reflect tissue of origin.

Limitations: Time-consuming, requires live animals/fresh tissue, and can have variability between preparations.

Types of Animal cell culture:

Type	Characteristics	Example
Adherent (Anchorage-dependent)	**Grow attached to vesse surface**	**Kidney, liver cells**
Suspension (Anchorage-independent)	**Float in medium**	**Blood cells (e.g., lymphocytes)**

Secondary Cultures/Cell Lines: Used to overcome the drawbacks of primary cultures.

- **Secondary Culture/Cell Line:** Result of subculturing a primary culture.
- **Subculturing (Splitting):** Periodic process of providing fresh nutrients and space for continuous growth.
- **Subculturing Procedure:** Involves removing old media, washing, dissociating cells (e.g., using trypsin (used first time by Rous and Jones), pipetting, or scraping), and diluting into fresh media.
- **Continuous Cell Lines:** Arise from spontaneous transformation in secondary cultures, exhibiting immortality (indefinite growth).
- **Cell Line Composition:** Can be mixed cell types or predominantly a single cell type.

Types of cell lines:

Feature	Finite Cell Lines	Continuous Cell Lines
Lifespan	Limited number of divisions	Indefinite (immortalized)
Contact Inhibition	Present	Lost
Anchorage Dependence	Required (anchorage-dependent)	Not required (anchorage-independent or both)
Ploidy	Diploid (normal chromosome number)	Aneuploid (abnormal chromosome number)
Growth Mode	Monolayer	Monolayer or Suspension
Growth Rate	Slow	Rapid
Doubling Time	24 to 96 hours	12 to 24 hours
Density Limitation	Present	Reduced or lost
Examples	Primary/early passage normal cells	HeLa, CHO cells

Essential Physical Environments: In vitro cell culture requires controlled physical, nutritional, and hormonal environments for cell growth.

- **Physical Environment Control:** Includes temperature, pH, osmolality, gaseous environment, support surface, and protection from stress.
- **Temperature:** Mammalian cells typically grow at **37°C** (core body temperature).
- **pH Regulation:** Crucial for ion balance, enzyme function, and hormone/growth factor binding. Media aim for **pH 7-7.4** using buffering systems.
- **Bicarbonate-CO2 Buffer:** A common buffering system used in cell culture media to maintain pH.

$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$

Addition of **$NaHCO_3$** helps neutralize acidic conditions

- **pH Indicator (Phenol Red):** Often included in media to visually monitor pH (yellow=acidic, pink=alkaline).
- **Osmolality:** Crucial for maintaining cell membrane integrity; typically around 300 mOsm, mainly determined by salts and glucose.
- **Growth Medium:** Mixture of inorganic salts and nutrients (amino acids, fatty acids, sugars, ions, trace elements, vitamins, cofactors) essential for cell survival and division.
- **Serum (e.g., FBS):** Important supplement providing growth factors, attachment factors, amino acids, hormones, lipids, vitamins, polyamines, and ions.
- **Trend Towards Serum-Free Media:** Due to complications associated with blood-based supplements.
- **Antibiotics:** Penicillin and streptomycin commonly added to control bacterial and fungal contamination (not essential for cell growth).

Equipment required for animal cell culture:

- **Culture Vessels:** Animal cells are grown in Petri dishes, flasks, or multi-well plates.
- **Incubation Conditions:** Typically 37°C and 5% CO2 for mammalian cells, but vary by cell type.
- **Aseptic Technique:** Crucial to prevent bacterial and fungal contamination.
- **Laminar Air Flow (LAF) Hoods:** Provide a sterile work environment, protecting both culture and operator. Air passes through HEPA filters, and UV light is used for sterilization.
- **CO2 Incubator:** Maintains sterility, constant temperature, fixed CO2 levels (5-10%), and high humidity (via water pan).
- **Centrifugation:** Low-speed centrifuges with gentle braking are used, often at 20°C.
- **Inverted Microscope:** Used to visualize cells at the bottom of culture vessels.

Applications of animal cell culture:

- Erythropoietin (EPO): Glycoprotein hormone stimulating RBC production and wound healing; recombinant human EPO (r-HuEPO) produced in CHO cells treats anemia. r-HuEPO Advantages: No donors/transfusion needed, no risk of transfusion-associated diseases.
- Factor VIII: Glycoprotein required for blood clotting; produced in CHO cells to treat Haemophilia A.
- Factor IX: Produced in CHO cells to treat Haemophilia B (Christmas disease).
- tPA (Tissue Plasminogen Activator): Serine protease dissolving blood clots; first drug produced via mammalian cell culture (in CHO cells).

Hybridoma Technology

- Antibodies: Bind specific antigen regions (epitopes).
- Polyclonal Antibodies: Heterogeneous mixture from different B-lymphocyte populations.
- Monoclonal Antibodies (mAbs): Highly specific, bind a single epitope. Useful for diagnostics and blocking.
- Hybridoma Technology: Fuses antibody-producing B cells with immortal myeloma cells using polyethylene glycol.

- Hybrid Cells (Hybridomas): Retain antibody secretion (B cell) and indefinite growth (myeloma).
- mAb Production: Hybridoma clones in culture produce epitope-specific mAbs.
- Impact: Revolutionized diagnostics and antibody-based therapies (e.g., early detection of hepatitis, AIDS).
- Developers: Cesar Milstein and George Kohler (Nobel Prize).

Therapeutic applications of mAb:

a) **OKT-3 (monab-CD3):** Immunosuppressant mAb, first used clinically.

- **Mechanism (OKT-3):** Blocks CD3 on T cells, inhibiting their function and reversing acute transplant rejection (heart, kidney, liver).
- **Reversibility (OKT-3):** T cell function returns to normal within a week after therapy.

b) **Herceptin (trastuzumab):** mAb for HER2+ early-stage breast cancer.

- **Mechanism (Herceptin):** Binds to HER2 receptors, blocking growth signals and impairing cancer growth.

Stem Cell Technology

- Stem Cell Definition: Self-renewing cells with the ability to differentiate into various specialized cell types.
- Analogy: Like good stock shares (self-renewal = bonus shares; differentiation = selling to buy goods).
- Importance: Maintain cell numbers in continuously renewing tissues (skin, blood, intestinal epithelium).
- Haematopoiesis: Well-studied process of blood cell formation (e.g., in human bone marrow).
- Key Researchers: Ernest McCulloch and James Till (1960s).

Types:

- Embryonic Stem (ES) Cells: Pluripotent (can differentiate into all cell types), from blastocysts.
- Adult Stem Cells: Multipotent (lineage-restricted), for tissue repair and maintenance.
- Applications: Grown and differentiated in culture for medical therapies.
- Therapeutic Potential: Treating leukemia, heart disease/attack, paralysis, Alzheimer's/Parkinson's/Huntington's, burns (replacing/repairing damaged/abnormal cells).

Embryonic Stem (ES) Cells:

- **Derivation:** Isolated from the inner cell mass (ICM) of embryos without immortalizing agents.
- **Culture:** ICM maintained with irradiated fibroblast cells.
- **Key Characteristics:**
  - Retain founder cell characteristics in culture.
  - Can reintegrate into embryogenesis.
  - Used to create chimeric mice (e.g., black ES cells in albino embryos yield black and white offspring).
  - Maintain a stable euploid karyotype.

- Can self-renew without differentiation in culture.

- **Genetic Manipulation:** Allows for gene knockouts (deleting a target gene from genome to understand its function) and precise genetic modifications to create mouse models of human diseases and search for new diagnostics and therapeutic modalities.

- **Human ES Cells:**
  - Isolated and grown in culture by James Thomson (1998).
  - Derived from ICM or human germ cells before meiosis.
  - Differentiated into specialized cells with specific growth factors (e.g., fibroblast growth factor, platelet-derived growth factor).

- **Therapeutic Potential:** Opens new possibilities for stem cell therapy in clinics.

MCQ

1. The differences in growth patterns between normal and cancer cells in culture are utilized in which diagnostic technique?

a) Karyotyping

b) Western blotting

c) **Colony formation assay**

d) ELISA

2. What is the primary risk associated with subculturing cells too frequently or at too low a density?

a) Increased risk of contamination.
b) Cell differentiation.
c) **Loss of the cell line.**
d) Accelerated cell senescence.

3. How does the inclusion of phenol red in many cell culture media demonstrate a practical application of understanding the medium's properties?

a) Phenol red directly contributes to cell growth by providing essential nutrients.
b) **Phenol red acts as a visual indicator, simplifying pH monitoring and maintenance of optimal cell culture conditions.**
c) Phenol red is essential for maintaining the correct osmolality of the medium.
d) Phenol red is a necessary cofactor for many enzymatic reactions within the cell.

4. Which of the following best explains the shift from blood-based supplements to serum-free media in animal cell culture?

a) Blood-based supplements are more expensive.
b) Serum-free media promotes faster cell growth.
c) **Blood-based supplements pose complications and risks.**
d) Serum-free media requires less maintenance.

5. Specific environmental conditions are maintained in a CO2 incubator. Which of these conditions is LEAST directly related to preventing cell death?

a) Maintaining sterility
b) Constant temperature
c) High relative humidity
d) **A fixed level of CO2**

6. Several therapeutic proteins are produced in CHO cells. What underlying characteristic of these proteins likely necessitates this choice of production system?

a) Their small size and simple structure.
b) Their susceptibility to degradation by bacterial enzymes.
c) Their complex glycosylation patterns and large size.
d) **Their requirement for specific post-translational modifications only found in mammalian cells.**

7. Which of the following statements best reflects the relationship between hypoxia and EPO production?
a) Hypoxia inhibits EPO production, leading to anemia.
b) **Hypoxia stimulates EPO production to increase oxygen-carrying capacity.**
c) EPO production is independent of oxygen levels in the body.
d) Hypoxia only affects EPO production in individuals with pre-existing anemia.

8. Recombinant human EPO (r-HuEPO) offers a significant advantage over blood transfusions primarily because:
a) It is more readily available.
b) It stimulates faster red blood cell production.
c) **It eliminates the risk of transfusion-associated diseases and the need for donors and facilities.**
d) It is a more cost-effective treatment option

9. What distinguishes monoclonal antibodies (mAbs) from polyclonal antibodies in terms of their specificity?
a) mAbs are produced from serum
b) mAbs bind to multiple epitopes on antigens
c) **mAbs bind specifically to a single epitope on an antigen**
d) mAbs are always produced in response to infectious diseases

10. What is the primary mechanism by which OKT3 prevents acute graft rejection?
a) Enhancing T cell activity
b) Blocking the function of CD4 molecules
c) **Inhibiting the function of T cells by binding to CD3**
d) Stimulating natural killer cells

11. How does Herceptin interfere with cancer cell growth?
a) It directly kills cancer cells
b) It enhances immune response against cancer cells
c) It induces apoptosis in cancer cells
d) **It blocks HER2 receptors from receiving growth signals**

12. Which of the following is NOT a key environmental parameter maintained by a CO2 incubator?

a) Constant temperature b) Fixed level of CO2

c) High relative humidity d) **Variable oxygen concentration**

13. What is the typical range of CO2 concentration used for culturing animal cells in a CO2 incubator?

a) 0-1% b) 2-4% c) **5-10%** d) 15-20%

14. Biosafety cabinets are classified from Class I to Class III primarily based on:

a) **The level of protection they provide to the user, the environment, and the product.**

b) The colour coding used for identification.

c) The size and dimensions of the cabinet.

d) The type of filtration system installed

15. What is the primary function of the High Efficiency Particulate Air (HEPA) filter in a Laminar Air Flow (LAF) hood?

a) To regulate the temperature of the airflow.

b) To remove harmful chemical vapors from the air.

c) **To eliminate particulate matter, such as microorganisms, from the air.**

d) To control the humidity levels within the hood.

16. Why is recombinant human EPO (r-HuEPO) considered advantageous compared to blood transfusions?
a) It can be produced from human donors
b) **It eliminates the need for donor availability and reduces the risk of disease transmission**
c) It increases the number of white blood cells in circulation
d) It is a more cost-effective treatment option for all medical conditions

17. What is the primary advantage of monoclonal antibodies in medical diagnostics?
a) They are easier to produce than polyclonal antibodies.
b) They can bind to multiple epitopes simultaneously.
c) **They provide epitope-specific binding for targeted diagnostics.**
d) They require less funding for research and development.

18. What are the specific regions on antigens that antibodies bind to?
a) Antigenic determinants b) **Epitopes**
c) Antigen binding sites d) Immunogenic regions

19. Which type of antibodies is known to be a heterogeneous population derived from various B lymphocyte populations?
a) Monoclonal antibodies b) **Polyclonal antibodies**
c) Recombinant antibodies d) Chimeric antibodies

20. What is the typical duration for T cell function to return to normal after OKT-3 therapy is discontinued?

a) Within 24 hours. b) Within 3-4 days.

c) **Within approximately one week.** d) Within one month.

21. OKT-3 (monab-CD3) is primarily used in transplant medicine for what purpose?

a) To prevent infections in immunocompromised patients.

b) To stimulate the growth of transplanted organs.

c) **To reverse acute rejection of transplanted organs.**

d) To enhance the body's natural immune response.

22. Herceptin's action directly targets:

a) DNA within the cancer cell nucleus.

b) **Cell surface receptors on cancer cells.**

c) Cytoplasmic enzymes involved in cell division.

d) The tumor microenvironment.

23. Herceptin (trastuzumab) is a therapeutic monoclonal antibody used for the treatment of which specific type of cancer?

a) **HER2-positive early-stage breast cancer** b) Melanoma

c) Lung cancer d) Leukemia

24. The ultimate outcome of Herceptin's mechanism of action is:

a) **Increased sensitivity of cancer cells to chemotherapy.**

b) Enhanced repair of damaged tissues surrounding the tumor.

c) Impaired growth of breast cancer cells.

d) Stimulation of the production of new HER2 receptors

25. Adult stem cells primarily function as:

a) The primary drivers of embryonic development.

b) **A repair system for the body, maintaining tissue turnover.**

c) The main source of immune cells in the body.

d) Regulators of metabolic processes in various organs.

26. What is the differentiation potential of embryonic stem (ES) cells?

a) Unipotent

b) Multipotent (lineage restricted)

c) **Pluripotent (can differentiate into all specialized tissues)**

d) Totipotent (can give rise to all cell types, including extraembryonic tissues)

27. Embryonic stem (ES) cells are derived from which stage of embryonic development?

a) Zygote b) Morula c) **Blastocyst** d) Gastrula

28. Where does haematopoiesis primarily occur in mice?

a) Liver and kidneys b) Brain and spinal cord

c) **Spleen and bone marrow** d) Thymus and lymph nodes

29. What is the approximate daily production of red blood cells (RBCs) by haematopoietic stem cells in a human being (per kg body weight)?

a) One million b) **One billion** c) One trillion d) One thousand

30. The experiment involving the injection of ES cells from a black mouse into the embryo of an albino mouse, resulting in progeny with black and white skin colour, demonstrates which property of ES cells?

a) Their ability to induce mutations in the host embryo.

b) Their potential to contribute to the development of various tissues in a developing organism.

c) **Their preference for integration into specific cell lineages.**

d) Their ability to alter the genetic makeup of the host.

31. The differentiation of human ES cells into specialized cell types in culture often requires:

a) Exposure to high levels of radiation.

b) **The presence of specific growth factors.**

c) Mechanical agitation of the culture dish.

d) Culturing at very low temperatures

ASSERTION REASON QUESTIONS

a) Both assertion and reason are true and the reason correctly explains the assertion.

b) Both assertion and reason are true but the reason does not explain the assertion.

c) Assertion is true and reason is false.

d) Both assertion and reason are false.

e) Assertion is false and reason is true.

1. Assertion: Embryonic stem (ES) cells are derived directly from the inner cell mass of growing embryos with the use of immortalizing agents.

Reason: The use of immortalizing agents could potentially maintain the fundamental properties and differentiation potential of the ES cells.

2. **Assertion:** Irradiated fibroblast cells are often used in the cell culture of the inner cell mass (ICM) of embryos.

Reason: Irradiated fibroblasts provide essential growth factors and a supportive matrix that helps maintain the undifferentiated state of the ES cells.

3. **Assertion:** Maintaining a stable euploid karyotype is crucial for embryonic stem (ES) cells in culture.

Reason: Changes in chromosome number or structure can occur spontaneously.

4. **Assertion:** Monoclonal antibodies (mAbs) are highly specific for a particular epitope on an antigen.

Reason: mAbs are produced by a single clone of immortalized B-lymphocytes, all secreting antibodies with the same antigen-binding site.

5. **Assertion:** Monoclonal antibodies have revolutionized the area of diagnostics.

Reason: Their high specificity allows for the precise detection of specific antigens, aiding in the early diagnosis of various diseases.

6. **Assertion:** Polyclonal antibodies present in serum are a heterologous population.

Reason: Polyclonal antibodies are released by the same populations of B-lymphocytes, each recognizing the same epitopes on the same antigen.

7. **Assertion:** OKT-3 (monab-CD3) is used to reverse acute rejection of transplanted organs.

Reason: OKT-3 is a monoclonal antibody.

8. **Assertion:** OKT3 (trastuzumab) is effective in treating HER2-positive breast cancer.

Reason: OKT3 to CD3 receptors on T cells, blocking their proliferation.

9. **Assertion:** Oncologists utilize the differences in growth patterns between normal and cancer cells in culture.

Reason: Assays like the colony formation assay exploit these differences to help determine if tumors are cancerous based on their ability to grow without contact inhibition.

10. **Assertion:** Normal animal cells in culture typically undergo a limited number of cell divisions.

Reason: This limited lifespan in culture reflects the inherent mortality associated with normal animal cells, similar to the finite growth of an organism to adulthood.

ANSWERS:

1	2	3	4	5	6	7	8	9	10
D	a	b	a	a	c	b	e	a	a

VERY SHORT ANSWER QUESTIONS:

1. a) What is the primary role of erythropoietin (EPO) in the body?

Answer: Stimulates red blood cell production.

b) Under what physiological condition is EPO production typically increased?

Answer: Hypoxia (shortage of oxygen).

2. a) Why is recombinant human EPO (r-HuEPO) considered advantageous over blood transfusion? Give one reason.

Answer: No risk of transfusion-associated disease.

b) What characteristic of EPO makes it relevant in cases of anemia associated with chronic renal failure?

Answer: It stimulates red blood cell production, which is often impaired in renal failure.

3. a) What type of cells is commonly used to produce recombinant glycoproteins like EPO and Factor VIII?

Answer: Chinese Hamster Ovary (CHO) cells.

b) What is the consequence of a deficiency in Factor IX?

Answer: Haemophilia B (or Christmas disease).

4. a) What is the enzymatic function of tissue plasminogen activator (tPA)?

Answer: Converts plasminogen to plasmin.

b) What is the therapeutic application of tPA?

Answer: Dissolving blood clots (e.g., in heart attack or stroke).

5. A researcher observes that their cell culture is growing in multiple layers and has a high cell density. Based on the information provided, is this more likely a finite or a continuous cell line? Justify your answer.

Answer: This is more likely a continuous cell line. The text states that continuous cell lines often lose contact inhibition and density limitation, allowing them to grow to high densities and potentially in multiple layers (though monolayer growth is also possible). Finite cell lines, in contrast, exhibit contact inhibition and density limitation, typically growing as a monolayer and stopping growth when they reach a certain density.

SHORT ANSWER QUESTIONS:

1. What are the key advantages of using human embryonic stem cells in research and potential therapies compared to using differentiated cells from adult tissues?

Answer: The key advantage of using human embryonic stem cells lies in their pluripotency – their ability to differentiate into all types of specialized tissues in the body. This broad potential makes them valuable for studying the development of various cell types and for potential therapies aimed at replacing or repairing damaged tissues in a wide range of diseases. In contrast, differentiated cells from adult tissues are already specialized and generally have a limited capacity to differentiate into other cell types, restricting their therapeutic applications. Additionally, ES cells can be grown and manipulated in culture to a greater extent while retaining their fundamental characteristics.

2. Explain the mechanism by which OKT3, a monoclonal antibody, acts as an immunosuppressant to prevent organ rejection. Based on this mechanism, why does T cell function typically return to normal after OKT3 therapy is discontinued?

Answer: OKT3 acts as an immunosuppressant by binding to the CD3 molecule on the surface of T cells, which are key players in acute graft rejection. This binding blocks the normal function of the T cells, thereby preventing them from attacking the transplanted organ. When OKT3 therapy is over, the existing OKT3 molecules gradually clear from the body. Since the T cells themselves are not permanently destroyed but only had their function blocked, new T cells without bound OKT3 can resume their normal immune functions within about a week.

3. Herceptin is a monoclonal antibody used to treat HER2-positive breast cancer. Based on the information provided, what is the role of the HER2 receptor in cancer cell growth, and how does Herceptin interfere with this process to inhibit cancer progression?

Answer: The HER2 receptor is a cell surface protein that normally receives growth signals, promoting cell growth and multiplication. In HER2-positive breast cancer, these receptors are often overexpressed, leading to excessive growth signaling and uncontrolled proliferation of cancer cells. Herceptin works by specifically binding to the HER2 receptors on the surface of these cancer cells. This binding blocks the receptors from receiving the growth signals, effectively disrupting the signaling pathway that drives cancer cell growth and thus inhibiting the progression of breast cancer.

4. A diagnostic test for a specific viral infection requires the ability to detect a unique protein on the surface of the virus. Would polyclonal or monoclonal antibodies be more suitable for this purpose? Justify your choice, considering the advantages and disadvantages of each type.

Answer: Monoclonal antibodies would be more suitable for this diagnostic test. Their high specificity for a single epitope on the viral protein ensures that the test is more likely to accurately identify the presence of the specific virus and reduce the chances of false positives due to binding to other similar proteins. While polyclonal antibodies might offer a stronger overall binding due to targeting multiple epitopes, their potential to cross-react with other antigens could lead to less reliable results in a diagnostic setting where precise detection is crucial.

5. Compare and contrast finite and continuous cell lines based on their lifespan in culture and their requirement for surface attachment.

Answer: Finite cell lines have a limited lifespan and will eventually stop dividing, typically exhibiting anchorage dependence (requiring a surface for growth). Continuous cell lines, on the other hand, are immortal and can grow indefinitely in culture, often losing anchorage dependence and being able to grow in suspension.

LONG ANSWER QUESTIONS

1. a) Explain the analogy of stem cells with stock to describe the dual nature of stem cells . How does this analogy help in understanding the key properties of stem cells?

Answer:" Self-renewal is likened to "multiplying" shares by receiving bonus shares, increasing the number of stem cells. Differentiation is compared to "selling" shares to "buy goods," where stem cells transform into specialized cell types with specific functions. This analogy effectively illustrates the stem cell's ability to both proliferate and diversify into various cell types, similar to how a stock can increase in quantity or be converted into other assets.

b) A researcher is studying a tissue that is constantly being regenerated. Based on the information provided, what type of stem cells would you expect to find in this tissue, and what is their primary role? Justify your answer.

Answer: We would expect to find adult stem cells in this constantly regenerating tissue. The text states that adult stem cells "act as a repair system for the body by maintaining the normal turnover of regenerative organs, such as, blood, skin, or intestinal tissues." Their primary role is to replenish the cells lost due to normal wear and tear or injury, ensuring the tissue maintains its functional capacity.

2. a) Consider a scenario where scientists introduce embryonic stem cells from a black mouse into the early embryo of a white mouse. What would be a likely characteristic of the resulting offspring's skin? Explain the scientific principle behind this observation.

Answer: The resulting offspring would likely have patches of both black and white skin, creating a chimeric appearance. This is because the introduced embryonic stem cells from the black mouse retain their genetic information and contribute to the development of various tissues, including the skin. Since some skin cells originate from the black mouse's ES cells and others from the white mouse's embryo, the offspring exhibits a mixture of both genetic origins in its skin.

b) Describe the process of "gene knockout" in mice using embryonic stem cells in general terms and explain why such mouse models are valuable in biomedical research.

Answer: Creating a "gene knockout" mouse involves selectively removing a specific gene from mouse embryonic stem cells through genetic manipulation. These modified ES cells are then introduced into mouse embryos, leading to the development of mice that lack a functional copy of the targeted gene. Such mouse models are invaluable in biomedical research because they allow scientists to study the function of the knocked-out gene and understand its role in normal development and disease processes. By observing the effects of the missing gene in a living organism, researchers can gain insights into the genetic basis of human diseases and test potential diagnostic and therapeutic strategies.

3. a) Explain the key difference between polyclonal and monoclonal antibodies in terms of their binding specificity to antigens. How does this difference arise from their cellular origins?

Answer: Polyclonal antibodies are a heterogeneous mixture that bind to multiple different epitopes on a single antigen because they are produced by various populations of B lymphocytes, each recognizing a different part of the antigen. Monoclonal antibodies, in contrast, are highly specific and bind to only a single epitope on an antigen. This specificity arises because they are produced by a single clone of immortalized B lymphocytes (hybridoma), all derived from one antigen-activated B cell that recognizes that particular epitope.

b) Describe the hybridoma technology developed by Milstein and Kohler. What are the essential characteristics of the hybrid cells that make this technology so valuable for producing monoclonal antibodies?

Answer: Hybridoma technology involves fusing an antibody-producing B lymphocyte (which has a limited lifespan) with a myeloma cell (a cancerous lymphocyte with the ability to grow indefinitely) using polyethylene glycol. The resulting hybrid cells, called hybridomas, possess two crucial characteristics: they retain the B cell's ability to secrete specific antibodies and the myeloma cell's ability to divide and grow continuously in culture. This combination allows for the large-scale production of a pure, epitope-specific monoclonal antibody.

4. a) Explain why enzymes like trypsin and collagenase are crucial in establishing a primary cell culture from a tissue sample.

Answer: These enzymes are used to break down the extracellular matrix and the proteinaceous connections that hold cells together in the tissue, allowing for the dissociation of individual cells needed to initiate the culture.

b) Differentiate between adherent and suspension cell cultures, relating their growth characteristics to the origin of the cells in the organism.

Answer: Adherent cells, typically derived from solid tissues, require attachment to a surface to grow, mimicking their anchorage within the extracellular matrix in vivo. Suspension cells, usually originating from the blood system, do not require attachment and grow freely in the culture medium, similar to how blood cells are suspended in plasma.

c) What is the primary purpose of subculturing cells in a continuous cell line? Describe one critical step involved in this process.

Answer: The primary purpose of subculturing is to provide fresh nutrients and more space for the continuously growing cells, preventing them from exhausting the medium and overcrowding. One critical step is dissociating the adhered cells from the culture vessel, often using enzymes like trypsin, to create a single-cell suspension that can be diluted and transferred to a new vessel with fresh medium.

5. a) Normal animal cells in culture divide and stop growing once they cover the surface of the vessel, relating this to the limited growth of an organism to adulthood. However, individual cells within an adult organism, like liver cells after injury, *can* proliferate. How can you reconcile this apparent contradiction between the behavior of cells in culture and in a living organism?

Answer: The "contact inhibition" observed in cell culture reflects a regulatory mechanism that prevents overgrowth in a confined two-dimensional space. While a mature organism's overall size is limited, individual cells within it retain the capacity to divide for repair and regeneration in response to specific signals (like tissue damage) within a complex three-dimensional environment regulated by various growth factors, hormones, and cell-cell interactions that are not fully replicated in standard cell culture. Contact inhibition in culture is one aspect of growth regulation, but in vivo, many other factors modulate cell proliferation.

b) If you were to perform a "colony formation assay" with both normal and cancerous cells, what key visual difference in the resulting colonies would allow you to distinguish between them, and what underlying principle of cell behavior would this difference reflect?

Answer: In a colony formation assay, normal cells would likely form a monolayer of cells that eventually stops growing once the cells make contact with each other, resulting in a relatively even distribution across the growth surface. Cancerous cells, having lost contact inhibition, would continue to proliferate even after contact, leading to the formation of dense, multi-layered clumps or colonies of cells piling on top of each other. This visual difference reflects the fundamental difference in their growth regulation: normal cells exhibit controlled proliferation influenced by cell-cell contact, while cancerous cells demonstrate uncontrolled growth independent of these signals.

CASE BASED QUESTIONS

1. Cells comprising tissues and organs such as the liver grow only to a certain size after which they cease to grow. This phenomenon which occurs in the normal body is observed also in cell culture and is termed "contact inhibition". This means that when cells grow and reach the walls of the container (i.e., reach confluency) they stop growing further. Another important feature of cell growth in culture is that their environment is different from that in vivo. These differences affect the adherence of cells to culture vessels, their shape and rate of proliferation. It is of interest to know that in culture, cancer cells appear very different from normal cells. Cancer cells lose contact inhibition and pile on each other due to uncontrolled growth and among other features, appear more rounded in shape.

a) The text highlights that the in vitro environment differs from the in vivo environment, affecting cell behavior such as adherence, shape, and proliferation rate. If a researcher observes that their cultured cells are exhibiting a significantly different morphology and growth rate compared to what is described for those cells within the organism, what are two potential explanations for these discrepancies based on the provided information? Answer: Two potential explanations are:

- **Lack of appropriate environmental cues:** The culture medium and vessel surface may not be providing the necessary signals (e.g., specific growth factors, extracellular matrix components, cell-cell interactions) that the cells would normally experience in the complex in vivo environment, thus altering their adherence, shape, and proliferation.
- **Transformation or selection of altered cells:** Over time in culture, some cells may undergo spontaneous transformation that alter their characteristics, or the culture conditions might selectively favor the growth of a subpopulation of cells with different properties than the original tissue.

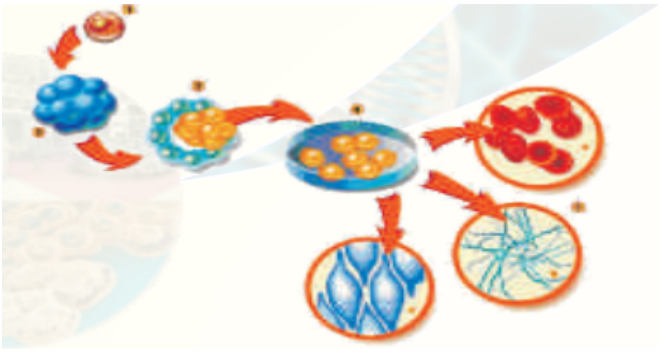
b) Normal animal cells in culture have a limited number of generations they can undergo, linked to a "mortality associated with all normal animal cells," whereas cancer cells can become immortal. Considering this, if a researcher establishes a primary cell culture and observes that the cells continue to proliferate indefinitely without any signs of slowing down or senescence, what might be a plausible explanation for this observation, even if the original tissue was non-cancerous?

Answer: A plausible explanation is that some cells in the primary culture have undergone spontaneous transformation in vitro, acquiring the characteristics of immortality seen in cancer cells. This transformation could involve genetic mutations or epigenetic changes that bypass the normal cellular mechanisms limiting cell division, leading to the establishment of a continuous cell line from what was initially a normal primary culture.

c) The text draws a parallel between contact inhibition in cell culture and the limited growth of an animal to adulthood. However, within an adult animal, cell division is still crucial for tissue repair and replacement. How does the concept of "contact inhibition" in vitro relate to the regulated cell division that occurs for homeostasis in a mature organism, where cells are in constant contact?

Answer: While contact inhibition in vitro demonstrates a basic mechanism of growth regulation based on cell-cell contact, the regulated cell division in a mature organism for homeostasis is a more complex process involving a delicate balance of positive and negative signals. Even when cells are in contact, specific growth factors, cytokines, and signaling pathways can override contact inhibition to allow for controlled proliferation in response to tissue damage or normal cell turnover. Contact inhibition in culture provides a simplified model of one aspect of growth control but doesn't fully encompass the intricate regulatory networks operating within a living organism.

CASE 2. Stem cells are characterized by their ability to renew themselves through mitotic cell division and differentiate into a diverse range of specialized cell types. Stem cells are found in all multicellular organisms. Stem cells are like good shares in the stock market which can either be multiplied (self renewal) by getting bonus shares or sold to buy goods (differentiate). Tissues like skin, blood and intestinal epithelium are subject to continuous renewal throughout life and must maintain an adequate number of cells (stem cells) that retain the potential to proliferate to make good such losses. The most well studied process has been the formation of blood cells (haematopoiesis). It was known in the case of mice that haematopoiesis occurs in the spleen and bone marrow. In human beings about 100,000 haematopoietic stem cells produce one billion RBC, one billion platelets, one million T cells, one million B cells per Kg body weight per day. The field of stem cell research was established in the 1960s by Ernest McCulloch and James Till at the University of Toronto.



A. What is depicted in the above given image?

B. Name two diseases which can be treated with stem cell therapy.

C. Write two sources of stem cells in an adult human being

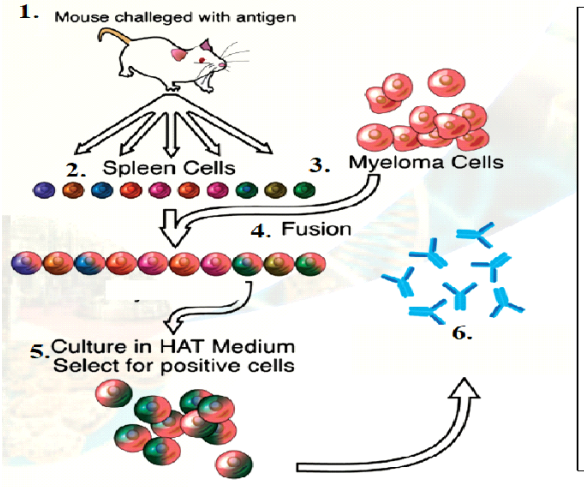
D. Differentiate between pluripotent stem cell and a multipotent stem cell.

Answers

1. Cultivation of embryonic stem cells
2. Leukemia, partalysis, burns
3. Bone marrow, spleen
4. Pluripotent cells: ES cells are pleuripotent and can differentiate into all types of specialized tissues. The adult stem cells are multipotent (lineage restricted) and act as a repair system for the body by maintaining the normal turnover of regenerative organs, such as, blood, skin, or intestinal tissues

CASE 3. Hybridoma technology is one of the most common methods used to produce monoclonal antibodies. In this process, antibody-producing B lymphocytes are isolated from mice after immunizing the mice with specific antigen and are fused with immortal myeloma cell lines to form hybrid cells, called hybridoma cell lines. These hybridoma cells are cultured in a lab to produce monoclonal antibodies, against a specific antigen. This can be achieved by an in vivo or an in vitro method. It is preferred above all the available methods to produce monoclonal antibodies because antibodies thus produced are of high purity and are highly sensitive and specific.

Production of monoclonal antibodies



a. In the technique depicted, the technician forgot step 5. What would be the outcome of the procedure? (1)

b. Name the fusogen used in step 4. (1)

c. What desired features of the Spleen cells and Myeloma cells are retained by the hybrid cells? (1)

d. Name a therapeutic agent that is produced using this technique and explain its use. (2)

Answers

1. HAT medium selects the fused cells of myeloma and spleen cells
2. Poly ethylene glycol is fusogen
3. Production of antibodies in a continuous fashion
4. Monoclonal antibody

CASE 4. The various types of cell lines are categorized into two types, i.e., finite cell line and continuous cell line. Finite cell lines are those cell lines which have a limited life span and they grow through a limited number of cell generations. Finite cell lines show the property of contact inhibition, density limitation and anchorage dependence. The mode of growth is in the monolayer form. The growth rate is slow and doubling time is around 24 to 96 hours.

Cell lines transformed under *in vitro* culture conditions give rise to continuous cell lines (Fig. 1). The various properties associated with continuous cell lines are: the ploidy (change in basic number of chromosomes), no contact inhibition and no anchorage dependence. The mode of growth is either monolayer or suspension form. The growth rate is rapid and doubling time is between 12 to 24 hours. The density limitation is reduced or lost.

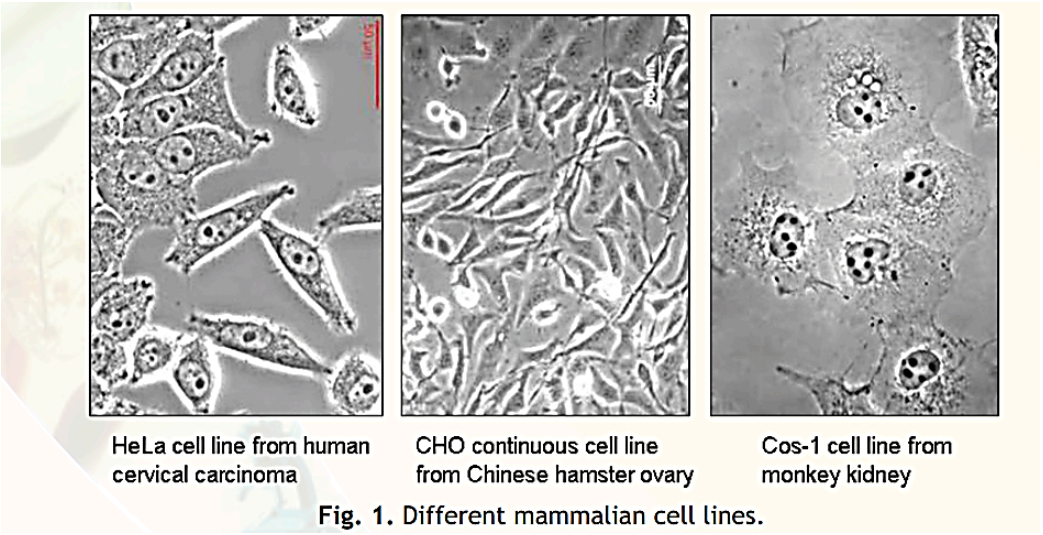


Fig. 1. Different mammalian cell lines.

1. Define contact inhibition

2. Name two properties of infinite cell line

3. Why continuous cell lines (myeloma) are preferred in monoclonal antibody production?

4. Define ploidy.

Answers

1. Contact inhibition is where cells stop growing once it touches the surface of a vessel.

2. Finite cell lines are those cell lines which have a limited life span and they grow through a limited number of cell generations. Finite cell lines show the property of contact inhibition, density limitation and anchorage dependence

3. Continuous cell lines are transformed and its fused with B cells in hybridoma technology to form monoclonal antibody

4. Change in basic number of chromosomes is called ploidy

CASE 5. OKT-3 is monab-CD3, an immunosuppressant drug given intravenously to reverse acute rejection of transplanted organs such as heart, kidney and liver. OKT3 is the first monoclonal antibody to be used for the treatment of patients. OKT3 acts by blocking the function of T cells which play a major role in acute graft rejection (Fig. 8). OKT3 binds and blocks the function of a cell surface molecule called CD3 in T cells. The binding of OKT3 to T cells results is followed by blocking of their functions. After OKT3 therapy is over, T cell function usually returns to normal within a week

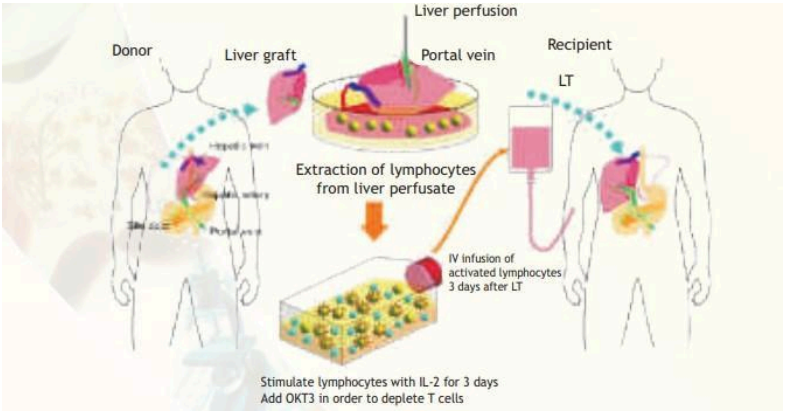


Fig. 8. Adoptive immunotherapy with OKT3 during liver transplantation (LT).

1.Which monoclonal antibody is immunosuppressant?

2. Which organs are generally transplanted in case of organ failure?

3.How does OKT3 act?

4. Which cells act as the first line of defense in our body?

Answers

1.OKT3 is an immunosuppressant.

2. Heart, kidney and liver can be transplanted in case of organ failure.

3. OKT3 acts by blocking the function of T cells which play a major role in acute graft rejection. OKT3 binds and blocks the function of a cell surface molecule called CD3 in T cells. The binding of OKT3 to T cells results is followed by blocking of their functions. After OKT3 therapy is over, T cell function usually returns to normal within a week

4. T cells are the first line of defense in our body.

CASE 6. The ES cells are cell lines derived directly from the inner cell mass of growing embryos without use of immortalising or transforming agents. The inner cell mass (lCM) of embryos can be maintained in cell culture in the presence of irradiated fibroblast cells.

The stem cells: a. retain the characteristics of founder cells, even after prolonged culture and extensive manipulation. b. reintegrates fully into embryogenesis if transferred. c. could be used to create chimeric mice by taking ES cells from a black mouse and implant it into the embryo of an albino mouse (white). The progeny so developed had skin color of black and white (a chimera Fig. 11). d. could maintain a stable euploid karyotype. e. could self-renew without differentiating in culture.

Now it is possible to selectively remove a gene (gene knock outs) and make other precise genetic modifications in the mouse ES cells and create mouse models of human diseases. Such mouse models have been extremely useful not only in understanding the genetic basis of a disease but also in search for new diagnostic and therapeutic modalities. In 1998, James Thomson developed a technique to isolate and grow human ES cells in culture. The human ES cells can be derived from the inner cell mass of blastocyst or from human germ cells before they initiate meiosis and cultured in a petri dish. Specialized cells can be grown in the presence of specific growth factors such as fibroblast growth factor and platelet-derived growth factor. The human ES cells have opened new possibilities for stem cell therapy in clinics.

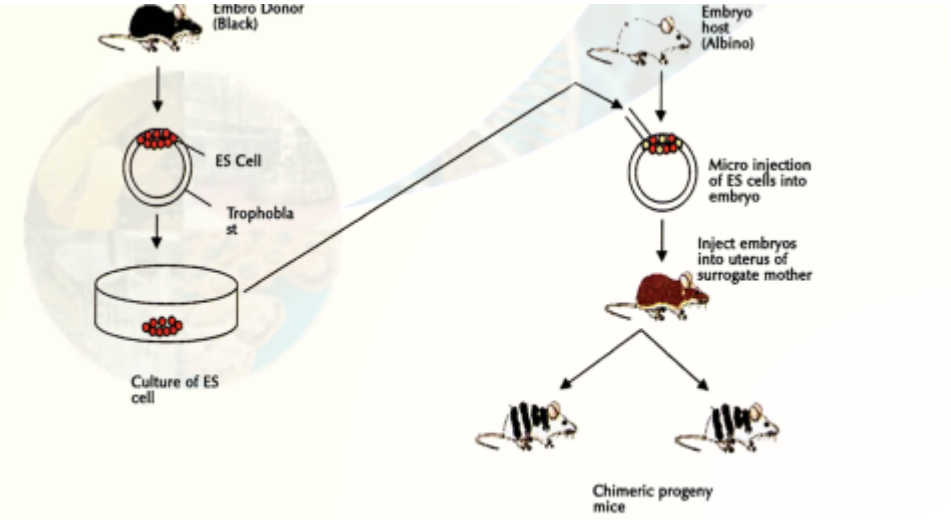


Fig. 11. Creation of chimeric mice using ES cells

1. **How are ES cell lines formed?**
2. **How are ICM Cells maintained?**
3. **Mention any two characteristics of stem cells.**
4. **What is a gene knock out?**

Answers

1. The ES cells are cell lines derived directly from the inner cell mass of growing embryos without use of immortalizing or transforming agents

2. The inner cell mass (lCM) of embryos can be maintained in cell culture in the presence of irradiated fibroblast cells

3. The stem cells:

a. retains the characteristics of founder cells, even after prolonged culture and extensive manipulation.

b. reintegrates fully into embryogenesis if transferred.

c. could be used to create chimeric mice by taking ES cells from a black mouse and implant it into the embryo of an albino mouse (white). The progeny so developed had skin color of black and white (a chimera)

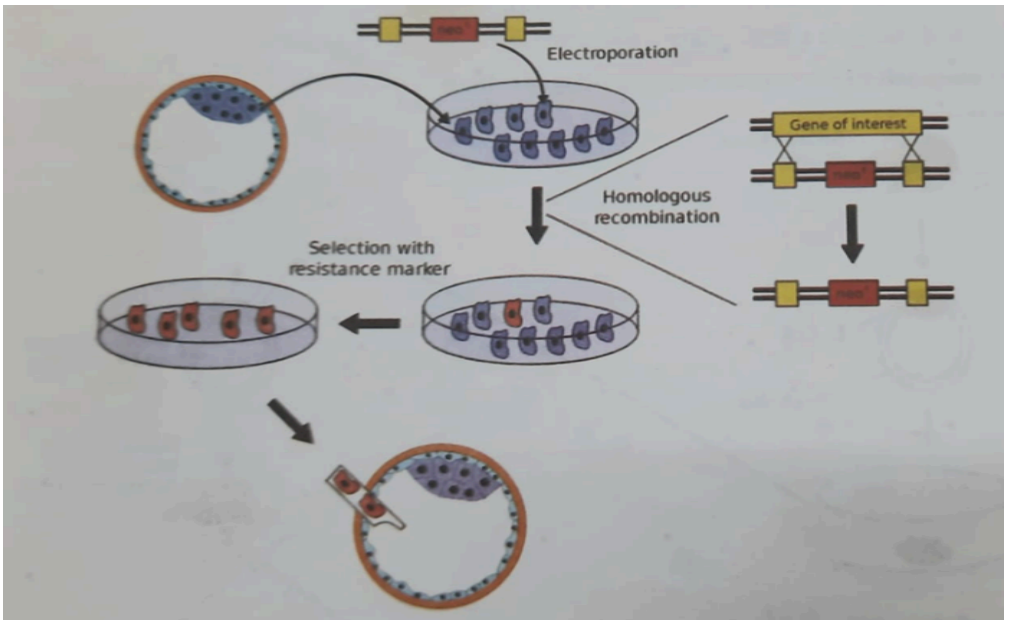
d. could maintain a stable euploid karyotype.

e. could self-renew without differentiating in culture. (any two)

4. Gene knock outs are where genes are selectively removed.

COMPETENCY BASED QUESTIONS

Observe the figure given below and answer the following questions-



a) What is the technique depicted here? 1

b) What is the principle behind the technique? 1

c) Expand the term ICM. From which stage of embryo is it extracted? 1

d) What is electroporation? 1

Answers

1. Homologous Recombination

2. Genetic recombination where genetic information is exchanged between two similar or identical double stranded DNA

3. Inner cell mass

4. Introduction of recombinant DNA to host cells using electrical field

Series : ZXW4Y

SET ~ 4

रोल नं.
Roll No.

प्रश्न-पत्र कोड
Q.P. Code **99**

परीक्षार्थी प्रश्न-पत्र कोड को उत्तर-पुस्तिका के मुख-पृष्ठ पर अवश्य लिखें ।
Candidates must write the Q.P. Code on the title page of the answer-book.

जैव-प्रौद्योगिकी
BIOTECHNOLOGY

निर्धारित समय : 3 घण्टे
Time allowed : 3 hours

अधिकतम अंक : 70
Maximum Marks : 70

नोट

(I) कृपया जाँच कर लें कि इस प्रश्न-पत्र में मुद्रित पृष्ठ **15** हैं ।

(II) कृपया जाँच कर लें कि इस प्रश्न-पत्र में **33** प्रश्न हैं ।

(III) प्रश्न-पत्र में दाहिने हाथ की ओर दिए गए प्रश्न-पत्र कोड को परीक्षार्थी उत्तर-पुस्तिका के मुख-पृष्ठ पर लिखें ।

(IV) **कृपया प्रश्न का उत्तर लिखना शुरू करने से पहले, उत्तर-पुस्तिका में यथा स्थान पर प्रश्न का क्रमांक अवश्य लिखें ।**

(V) इस प्रश्न-पत्र को पढ़ने के लिए 15 मिनट का समय दिया गया है । प्रश्न-पत्र का वितरण पूर्वाह्न में 10.15 बजे किया जाएगा । 10.15 बजे से 10.30 बजे तक परीक्षार्थी केवल प्रश्न-पत्र को पढ़ेंगे और इस अवधि के दौरान वे उत्तर-पुस्तिका पर कोई उत्तर नहीं लिखेंगे ।

NOTE

(I) Please check that this question paper contains **15** printed pages.

(II) Please check that this question paper contains **33** questions.

(III) Q.P. Code given on the right hand side of the question paper should be written on the title page of the answer-book by the candidate.

(IV) **Please write down the serial number of the question in the answer-book at the given place before attempting it.**

(V) 15 minute time has been allotted to read this question paper. The question paper will be distributed at 10.15 a.m. From 10.15 a.m. to 10.30 a.m., the candidates will read the question paper only and will not write any answer on the answer-book during this period. ~

General Instructions :

Read the following instructions carefully and follow them :

*(i) This Question paper contains **33** questions. **All** questions are **compulsory**.*

*(ii) Question paper is divided into **five** sections – Section **A**, **B**, **C**, **D** and **E**.*

*(iii) Section **A** : Questions number **1** to **16** are Multiple Choice Type Questions (MCQs). Each question carries **1** mark.*

*(iv) Section **B** : Questions number **17** to **21** are Very Short Answer (VSA) Type Questions. Each question carries **2** marks.*

*(v) Section **C** : Questions number **22** to **28** are Short Answer (SA) Type Questions. Each question carries **3** marks.*

*(vi) Section **D** : Questions number **29** and **30** are Case Study-based Questions, carrying **4** marks each. Each question has sub-parts with internal choice in one sub-part.*

*(vii) Section **E** : Questions number **31** to **33** are Long Answer (LA) Type Questions. Each question carries **5** marks.*

*(viii) There is no overall choice given in the question paper. However, an internal choice has been provided in few questions in all the Sections except Section – **A**.*

SECTION – A

1. The DNA ligase enzyme that is frequently used to ligate different DNA fragments in order to generate rDNA molecules is isolated from : 1

(A) T_2 Bacteriophage

(B) T_4 Bacteriophage

(C) Lambda Bacteriophage

(D) M13 filamentous phage

2. Protein Efficiency Ratio (PER) is used as a measure of growth expressed in terms of : 1

(A) Weight gain of an adult by consuming 1 g of food protein.

(B) Weight gain of an adult by consuming 100 g of food protein.

(C) Protein nitrogen that is retained by the body by consuming 1 g of food protein.

(D) Protein nitrogen that is retained by the body by consuming 100 g of food protein.

3. पीसीआर (PCR) के एक चक्र के अंत में डीएनए के दो अणु _____ हो जाएँगे । 1

(A) आठ (B) सोलह

(C) चार (D) दो

4. ApoE जीन में एकल क्षारक (बेस) का अंतर _____ से संबद्ध है । 1

(A) HIV (ह्यूमन इम्यूनोडिफिसिएंसी वायरस) के प्रति प्रतिरोधकता

(B) माइग्रेन

(C) हंटिंग्टन रोग

(D) अल्जाइमर रोग

5. सूक्ष्मजीवीय कोशिका संवर्धन में उपयोग किये जाने वाले कार्बन स्रोतों में से एक है 1

(A) ऑलिव ऑयल (B) अन्न के दानें

(C) अमोनियम लवण (D) वृद्धि कारक

6. एक जैवप्रौद्योगिकविज्ञ एक जीन अनुक्रम को एकल-रज्जुक रूप में प्राप्त करना चाहता है । वांछित परिणाम प्राप्त करने के लिए उसे किस जीवाणुभोजी आधारित संवाहक का उपयोग करना चाहिए ? 1

(A) M-13 आधारित संवाहक (B) लैम्डा आधारित जीवाणुभोजी संवाहक

(C) pUC कुल का प्लैज्मिड क्लोनिंग संवाहक (D) प्लैज्मिड संवाहक pBR 322

7. मानव में एडीनोसिन डिएमीनेज (एडीए) के अभाव के कारण होता है 1

(A) थैलैसीमिया (B) दात्र कोशिका अरक्तता

(C) एससीआईडी (SCID) (D) मैड काऊ रोग

8. मानव भ्रूण स्टेम कोशिकाओं का पृथक्करण तथा संवर्ध में संवर्धन की तकनीक विकसित करने वाले वैज्ञानिक का नाम है 1

(A) सीजर मिलस्टीन (B) जेम्स थॉमसन

(C) जॉर्ज कोहलर (D) जॉर्ज गे

9. जीवाणु के जीनोम के लिए जीन पूर्वानुमान _____ कम्प्यूटर प्रोग्राम के उपयोग द्वारा किया जा सकता है । 1

(A) जीनमार्क (B) GENSCAN

(C) UniProtKB (D) RefSeq

3. At the end of one cycle of PCR, two DNA molecules will become _____. 1

(A) Eight (B) Sixteen
(C) Four (D) Two

4. Single base difference in ApoE gene is associated with : 1

(A) Resistance to HIV (Human Immunodeficiency Virus)
(B) Migraine
(C) Huntington disease
(D) Alzheimer's disease

5. One of the carbon source used in microbial cell culture is : 1

(A) Olive oil (B) Cereal grains
(C) Ammonium salts (D) Growth factors

6. A biotechnologist wants to obtain a gene sequence in single stranded form. Which bacteriophage based vector should he choose to obtain the desired result ? 1

(A) M13 based vector
(B) Bacteriophage Lambda based vector
(C) Plasmid cloning vector of pUC family
(D) Plasmid vector pBR 322

7. Absence of the enzyme adenosine deaminase in humans causes : 1

(A) Thallasemia (B) Sickle cell anaemia
(C) SCID (D) Mad cow disease

8. The technique to isolate and grow human embryonic stem cells in culture was developed by : 1

(A) Ceasar Milstein (B) James Thomson
(C) George Kohler (D) George Gay

9. Gene prediction for bacterial genomes can be done by using the computer program _____. 1

(A) GeneMark (B) GENSCAN
(C) UniProtKB (D) RefSeq

10. _____ को पोषक (न्यूट्रास्यूटिकल) प्रोटीनों के आहार स्रोत के रूप में वर्गीकृत किया जा सकता है। 1

(A) बीज भण्डारित प्रोटीन
(B) सोया प्रोटीन
(C) दुग्ध प्रोटीन
(D) पनीर (छेने का) जल सांद्र

11. इस उपगमन (विधि) के उपयोग द्वारा रोग विशिष्ट प्रोटीनों की पहचान की जा सकती है : 1

(A) प्रकार्यात्मक प्रोटियोमिक्स
(B) संरचनात्मक प्रोटियोमिक्स
(C) अभिव्यक्ति प्रोटियोमिक्स
(D) प्रोटियोम माइनिंग

12. हरसेप्टिन एक एकक्लोनी ऐंटीबॉडी है जिसे स्वीकृत किया गया है 1

(A) स्तन कैंसर की प्रारंभिक अवस्था के उपचार हेतु
(B) निरोपित अंगों की तीव्र अस्वीकृति को विलोमित करने के लिए
(C) एरिथ्रोपोएसिस का उद्दीपन
(D) हृदयाघात वाले कुछ विशेष रोगियों के लिए उपयोग किया जाता है।

प्रश्न संख्या **13** से **16** के लिए, दो कथन दिए गए हैं – जिनमें एक को अभिकथन (A) तथा दूसरे को कारण (R) द्वारा अंकित किया गया है। इन प्रश्नों के सही उत्तर नीचे दिए गए कोडों (A), (B), (C) और (D) में से चुनकर दीजिए :

(A) अभिकथन (A) और कारण (R) दोनों सही हैं और कारण (R), अभिकथन (A) की सही व्याख्या करता है।
(B) अभिकथन (A) और कारण (R) दोनों सही हैं, परन्तु कारण (R), अभिकथन (A) की सही व्याख्या नहीं करता है।
(C) अभिकथन (A) सही है, परन्तु कारण (R) गलत है।
(D) अभिकथन (A) गलत है, परन्तु कारण (R) सही है।

13. **अभिकथन (A)** : CO_2 उष्मायित्र (इंक्यूबेटर) जन्तु कोशिका संवर्धन माध्यम की जलशुष्कन से रक्षा करते हैं।
कारण (R) : उच्च आपेक्षित आर्द्रता बनाए रखने के लिए CO_2 उष्मायित्र के अंदर हर समय पानी से भरा एक पात्र रखा जाता है। 1

14. **अभिकथन (A)** : अर्बुद (कैंसर) कोशिकाओं में संस्पर्श संदमन अनुपस्थित होता है।
कारण (R) : जब प्रसामान्य कोशिकाएँ पात्र की दीवार के संपर्क में आती हैं तो उनकी वृद्धि रुक जाती है। 1

10. _____ can be categorized as a food source of nutraceutical proteins. 1

(A) Seed storage proteins
(B) Soya protein
(C) Milk proteins
(D) Whey protein concentrates

11. Identification of disease specific proteins can be done by using the approach of : 1

(A) Functional proteomics
(B) Structural proteomics
(C) Expression proteomics
(D) Proteome mining

12. Herceptin is a monoclonal antibody approved for : 1

(A) Therapy of early stage breast cancer.
(B) Reversal of acute rejection of transplanted organs.
(C) Stimulation of erythropoiesis.
(D) Use in certain patients having a heart attack.

For Questions **13** to **16**, two statements are given – one labelled Assertion (A) and other labelled Reason (R). Select the correct answer to these questions from the codes (A), (B), (C) and (D) as given below :

(A) Both Assertion (A) and Reason (R) are true and the Reason (R) is the correct explanation of the Assertion (A).
(B) Both Assertion (A) and Reason (R) are true, but the Reason (R) is not the correct explanation of the Assertion (A).
(C) Assertion (A) is true, but Reason (R) is false.
(D) Assertion (A) is false, but Reason (R) is true.

13. **Assertion (A) :** CO_2 incubators prevent the desiccation of the animal cell culture medium.
Reason (R) : A pan of water is kept at all times in a CO_2 incubator chamber to maintain high relative humidity. 1

14. **Assertion (A) :** Contact inhibition is absent in cancer cells.
Reason (R) : Normal cells stop growing when they reach the walls of the container. 1

15. **अभिकथन (A)** : प्रोटीनों के विविध प्रकार्य हैं ।
कारण (R) : सभी प्रोटीन एंजाइम हैं । 1

16. **अभिकथन (A)** : दात्र कोशिका अरक्तता के आण्विक आधार का अध्ययन करने के प्रारंभिक प्रयासों में से एक प्रयास सामान्य हीमोग्लोबिन (Hb) तथा दात्र कोशिका हीमोग्लोबिन (scHb) के वैद्युत कण-संचलन की गतिशीलता की तुलना करना था ।
कारण (R) : वैद्युत कण-संचलन के दौरान दात्र कोशिका हीमोग्लोबिन (scHb) सामान्य हीमोग्लोबिन (Hb) की अपेक्षा अधिक तीव्रता से गति करती है । 1

खण्ड – ख

17. (a) संहति स्पेक्ट्रोमिति का सिद्धान्त क्या है ? इसके महत्त्वपूर्ण उपयोग लिखिए । 2

अथवा

(b) काइमोट्रिप्सिन एंजाइम में कार्यशील चार्ज रिले प्रणाली का संक्षिप्त विवरण लिखिए । 2

18. एक समुचित उदाहरण की सहायता से संक्षेप में व्याख्या कीजिए कि किसी जीव में पूर्वानुमानित जीनों की संख्या जीनोम आकार (साइज़) तथा गुणसूत्रों की संख्या के साथ सहसंबंध क्यों नहीं दर्शाते । 2

19. 'चिरकालिक मज्जाजनित श्वेतरक्तता' (क्रॉनिक मायलोजनिक ल्यूकेमिया) होने का क्या कारण है ? उस तकनीक का नाम लिखिए जिससे इस रोग की सही अवस्था को जाना जाता है । 2

केवल दृष्टिबाधित परीक्षार्थियों के लिए प्र. 19 का विकल्प

19. 'RefSeq database' में दिए गए आँकड़ों के क्या उपयोग हैं ? 2

20. एशरिकिया कोलाई से पुनर्योगज इंसुलिन (ह्यूमुलिन) के पृथक्करण के प्रमुख चरणों की रूपरेखा लिखिए । 2

21. सूक्ष्मजीवी संवर्धन माध्यम में फेनन का सर्वसामान्य कारण क्या है ? सूक्ष्मजीवीय प्रक्रमों में फेनन से कौन सी समस्यायें उत्पन्न होती हैं ? 2

15. **Assertion (A) :** Proteins have diverse functions.
Reason (R) : All proteins are enzymes. 1

16. **Assertion (A) :** One of the first attempts to study the molecular basis of sickle cell anaemia was to compare the electrophoretic mobility of normal haemoglobin (Hb) and sickle cell haemoglobin (scHb).
Reason (R) : Sickle cell haemoglobin (scHb) moved faster than normal haemoglobin (Hb) in electrophoresis. 1

SECTION – B

17. (a) What is the principle of mass spectrometry ? Write its important application. 2

OR

(b) Describe briefly, the charge relay system that operates in chymotrypsin enzyme. 2

18. With a suitable example, explain briefly, why the number of predicted genes do not correlate with the genome size and the number of chromosomes in an organism. 2

19. Why is chronic mylogenous leukemia caused ? Name the technique used to know the status of this disease. 2

Alternative Question for Visually Impaired in lieu of Q. No. 19.

19. What are the uses of the data provided in RefSeq database ? 2

20. Outline the important steps for isolation of recombinant insulin (Humulin) from Escherichia coli. 2

21. What is the most common cause of foaming in microbial culture medium ? Which problems are created by foaming in microbiological processes ? 2

✡ ✡

खण्ड – ग

22. पनीर/छेना जल की चिकित्सीय उपयोगिता की वैज्ञानिक प्रासंगिकता की व्याख्या समुचित उदाहरण की सहायता से कीजिए। 3

23. (a) निम्नलिखित संवाहकों में से प्रत्येक में समाविष्ट किए गए महत्त्वपूर्ण अभिलक्षणों का उल्लेख कीजिए : 3

(i) कॉस्मिडस्

(ii) शटल संवाहक

(iii) अभिव्यक्ति (एक्सप्रेशन) संवाहक

अथवा

(b) वांछित जीन संवाहक प्लैज्मिड युक्त पुनर्योगज कोशिकाओं की विविक्तिकर निरीक्षण (स्क्रीनिंग) के लिए नीले-श्वेत वरणात्मक तकनीक की व्याख्या कीजिए। 3

24. ऊतक प्लैज्मिनोजन सक्रियक (tPA) की क्रियाविधि (कार्य-प्रणाली) क्या है ? स्तनधारियों के कोशिका संवर्धन के द्वारा tPA निर्माण की विधि दर्शाने के लिए एक योजनात्मक आरेख बनाइए। 3

केवल दृष्टिबाधित परीक्षार्थियों के लिए प्रश्न 24 का विकल्प

24. "मूल कोशिकाएँ (स्टेम सेल्स)" क्या हैं ? दो मुख्य प्रकार की स्तनधारीय मूल कोशिकाओं (स्टेम सेल्स) के नाम लिखकर प्रत्येक का एक-एक उपयोग भी लिखिए। 3

25. यद्यपि कोशिकाओं की वृद्धि के लिए आवश्यक नहीं है फिर भी प्रतिजैविकों को जन्तु कोशिका संवर्धन माध्यम में निवेशित किया जाता है। इसका कारण लिखिए। ऐसे दो प्रतिजैविकों के नाम भी लिखिए। 3

26. अंतर्जातीय (इंटरस्पीशीज़) तथा अंतर्वंशीय संकर निर्मित करना अत्यंत कठिन है, क्यों ? इस प्रकार के अभिनव (नवल) संकर प्राप्त करने की तकनीक का संक्षिप्त वर्णन कीजिए। 3

27. पारजीनी पौधों का उपयोग पॉलीहाइड्रॉक्सीब्यूटिरेट (PHB) का वृहद स्तर पर उत्पादन करने के लिए फैक्ट्रीयों के रूप में किया जा सकता है। इस कार्य के लिए ऐराबिडोप्सिस पौधे को किस प्रकार अभियांत्रित (इंजीनियर्ड) किया गया ?

ऐल्केलीजीन यूट्रोफस जीवाणु का उपयोग करके किण्वन द्वारा PHB के उत्पादन में अवरोधों (कमियों) का उल्लेख कीजिए। 3

SECTION – C

22. Elaborate upon the scientific relevance for therapeutic usefulness of whey with specific examples. 3

23. (a) Mention the important features that were incorporated in each of the following vectors : 3
    (i) COSMIDS
    (ii) Shuttle Vectors
    (iii) Expression Vectors

OR

(b) Explain the method of Blue-White selection used for screening of recombinant cells containing desired plasmid with gene of interest. 3

24. What is the mode of action of tissue Plasminogen Activator (tPA) ? Draw a schematic representation to show the method of production of tPA through mammalian cell culture. 3

Alternative Question for Visually Impaired in lieu of Q. No. 24

24. What are stem cells ? Write the two broad types of mammalian stem cells and an application of each. 3

25. Although not required for cell growth, antibiotics are added in animal cell culture medium. Give reason. Name two such antibiotics. 3

26. It is very difficult to produce hybrids in case of interspecific and intergeneric crosses. Why ? Briefly describe the technique to obtain such novel hybrids. 3

27. Transgenic plants can be used as factories to produce polyhydroxybutyrate (PHB) on a large scale. How was Arabidopisis plant engineered for the same ?
    State the drawback of producing PHB by fermentation using bacterium Alcaligenes eutrophus. 3

28. प्रसामान्य कोशिकाओं तथा अर्बुद (कैंसर) कोशिकाओं के बीच एक तुलनात्मक सूक्ष्मव्यूह संकरण प्रयोग किया गया । यदि cDNA प्रोब्स को लेबल करने के लिए लाल तथा हरे वर्ण (रंग) के प्रदीप्त का उपयोग क्रमश: प्रसामान्य कोशिकाओं तथा कैंसर कोशिकाओं के लिए किया गया हो तो सूक्ष्मव्यूहन तकनीक से प्राप्त परिणामों की व्याख्या आप किस प्रकार करेंगे ? 3

खण्ड – घ

निर्देश : प्रश्न संख्या **29** तथा **30** प्रकरण (केस) आधारित प्रश्न हैं । प्रत्येक प्रश्न के **तीन** उपप्रश्न [(i), (ii) तथा (iii)] हैं तथा **एक** उपप्रश्न में आंतरिक विकल्प दिया गया है ।

29. किसी डीएनए खंड के न्यूक्लियोटाइड अनुक्रम को ज्ञात करने के लिए आमतौर पर सेंगर की डीएनए अनुक्रमन तकनीक (विधि) का उपयोग किया जाता है । डीएनए के एक रज्जुक टेम्पलेट को एक पथप्रदर्शक की तरह उपयोग करके प्राइमर्स का विस्तारण होता है, जहाँ सामान्य क्रियाधार, उदाहरणत: डीऑक्सीन्यूक्लियोटाइड 5' ट्राइफॉस्फेट्स (dNTPs) वर्धन करती डीएनए शृंखला में समावेशित हो जाते हैं । यह विधि ddNTPs (2′, 3′ डाईडीऑक्सीन्यूक्लियोटाइड ट्राइफॉस्फेट्स) के द्वारा शृंखला के समापन के सिद्धान्त पर आधारित है । जो यदि प्रत्येक वर्धनशील शृंखला में (वांछित dNTP के स्थान पर) समावेशित हो जाए, तो शृंखला का समापन हो जाता है । जेल स्वविकिरणी चित्रण द्वारा विलगित पृथक्कृत रज्जुकों को देखने के लिए विकिरण सक्रिय प्राइमर्स का उपयोग किया जा सकता है । आजकल डीएनए अनुक्रमन स्वचालित हो गया है जहाँ ddNTPs फ्लुओरेसेंट अणुओं के साथ जुड़ जाते हैं तथा प्राप्त जेल का लेज़र द्वारा क्रमवीक्षण (स्कैन) किया जाता है ।

(i) सेंगर की डीएनए अनुक्रमन तकनीक (विधि) में प्रारंभकों (प्राइमर्स) की आवश्यकता क्यों होती है ? 1

(ii) dNTPs तथा ddNTPs के बीच अंतर लिखिए । सेंगर की डीएनए अनुक्रमन विधि में ddNTPs का क्या कार्य है ? 2

(iii) डीएनए अनुक्रमन की स्वचित्रण विधि (तकनीक) में ddNTPs के फ्लुओरेसेंट अणुओं के संयुक्त संयोजित का उपयोग करने का क्या लाभ है ? 1

अथवा

(iii) सेंगर की डीएनए अनुक्रमन विधि में किस एंजाइम का उपयोग किया जाता है ? 1

28. A comparative microarray hybridization experiment was performed between normal and cancerous cells. How will you interpret the result obtained on a microarray, if red and green coloured fluors were used for labelling the cDNA probes of normal and cancerous cells respectively ? 3

SECTION – D

Instructions : Q. Nos. **29** and **30** are case-based questions. Each of these questions have sub-parts [(i), (ii) and (iii)] with internal choice in one sub-part.

29. Sanger's method of DNA sequencing is a widely used technique to determine the nucleotide sequence of a DNA fragment. Primers are extended using the single strand DNA template as a guide, where the normal substrates i.e. deoxynucleotide 5′ triphosphates (dNTPs) are incorporated in the growing DNA chain. This method is based upon the principle of chain termination by ddNTPs (2′, 3′ dideoxynucleotide tri phosphates) which, if incorporated into each extending chain (instead of the required dNTP) cause termination. Radioactive primers may be used to visualize separated strands in the gel by autoradiography. Nowadays, DNA sequencing have become automated, where ddNTPs are conjugated with fluorescent molecules and the gels obtained are scanned by laser.

(i) Why are primers required in Sanger's method of DNA sequencing ? 1

(ii) Write the difference between dNTPs and ddNTPs. What is the function of ddNTPs in Sanger's method of DNA sequencing ? 2

(iii) What is the advantage of using ddNTPs conjugated with fluorescent molecules in automated method of DNA sequencing ? 1

OR

(iii) Which enzyme is used in Sanger's method of DNA sequencing ? 1

30. कोशिका तथा ऊतक संवर्धन तकनीक के उपयोग द्वारा विजातीय जीनों के निवेशन द्वारा सुधरे सस्य विशेषकों वाली आनुवंशिकत: रूपांतरित अथवा पारजीनी फ़सलों को विकसित किया गया है । समुचित संवर्धन माध्यम में कर्तोतकों का संवर्धन किया जाता है । यह संवर्धन माध्यम स्थूल पोषकों, सूक्ष्मपोषक, कार्बन स्रोत, विटामिन, एमीनो अम्लों के साथ पादप कोशिकाओं के विभाजन तथा वृद्धि के लिए आवश्यक पादप हॉर्मोन प्रदान करता है । पादप कोशिका संवर्धन अनेक प्रकार के संवर्धन जैसे कि, अंग संवर्धन, कर्तोतकी संवर्धन, कैलस संवर्धन, कोशिका निलंबन संवर्धन, जीवद्रव्य (प्रोटोप्लास्ट) संवर्धन, मास सेल संवर्धन से संबंधित है और इसमें से प्रत्येक की पादप पुनर्जनन, आनुवंशिक रूपांतरण अध्ययन तथा पादप कोशिका एवं ऊतक संवर्धन के क्षेत्र में अनेक उपयोग हैं ।

(i) कर्त्तोतकी संवर्धन का क्या अभिप्राय है ? 1

(ii) पादप कोशिका संवर्धन में कोशिकाओं के विभाजन तथा वृद्धि को प्रोत्साहित करने वाले हॉर्मोनों का उल्लेख कीजिए । 2

(iii) "जीवद्रव्यक (प्रोटोप्लास्ट)" का क्या अर्थ है ? 1

अथवा

(iii) 'कैलस' संवर्धन का एक उपयोग लिखिए । 1

खण्ड – ङ

31. (a) दात्र कोशिका हीमोग्लोबिन की तुलना प्रसामान्य हीमोग्लोबिन से करने के लिए उपयोग की जाने वाली पेप्टाइड मानचित्रण (मैपिंग) तकनीक की व्याख्या कीजिए । 5

अथवा

(b) प्रोटीन आधारित पाँच उत्पादों का उल्लेख प्रत्येक के एक-एक समुचित उदाहरण के साथ कीजिए । 5

32. (a) पुनर्योगज डीएनए तकनीक (प्रौद्योगिकी) के मूल चरणों का वर्णन कीजिए । 5

अथवा

(b) प्रतिबंधन खंड लम्बाई बहुरूपता (रेस्ट्रिकशन फ्रेगमेंट लेंथ पॉलीमोरफिज्म – RFLP) तकनीक की कार्यविधि का वर्णन कीजिए । विधि विज्ञान में इस तकनीक का उपयोग क्यों किया जाता है ? 5

33. (a) "मेटाजीनोम" क्या है ? मेटाजीनोमिक्स उपगमन का वर्णन कीजिए तथा सूक्ष्मजीवों के अध्ययन में इसका महत्त्व लिखिए । 5

अथवा

(b) एक सूक्ष्मजीवी संवर्धन में सतत् संवर्धन तंत्र किस प्रकार अनुरक्षित रखा जाता है ? फेड-बैच संवर्धन की अपेक्षा इसके क्या लाभ हैं ? 5

30. Genetically modified or transgenic crops with improved agronomic traits have been developed by introduction of foreign genes into crop plants, using cell and tissue culture systems. The explants are cultured on a suitable nutrient medium which provides macro-nutrients, micronutrients, carbon source, vitamins, amino acids alongwith plant hormones required for growth, cell division and development of plant cells in culture. Plant cell culture and applications deal with various types of cultures such as organ culture, explant culture, callus culture, cell suspension culture, protoplast culture, mass cell culture and each of these have widespread uses in plant regeneration, genetic transformation studies, and many more applications of plant cell and tissue culture.

(i) What is meant by explant culture ? 1

(ii) Mention hormones that are used for promoting growth and cell division of plant cells in culture. 2

(iii) What is meant by "Protoplast" ? 1

OR

(iii) Write an application of callus culture. 1

SECTION – E

31. (a) Explain the technique of peptide mapping used to compare normal haemoglobin with sickle cell haemoglobin. 5

OR

(b) Mention any five protein based products with an example of each. 5

32. (a) Describe the basic steps of Recombinant DNA Technology. 5

OR

(b) Describe the procedure involved in Restriction Fragment Length Polymorphism (RFLP) technique. Why is this technique used in forensic sciences ? 5

33. (a) What is a "metagenome" ? Describe the metagenomics approach and write its importance to study microorganisms. 5

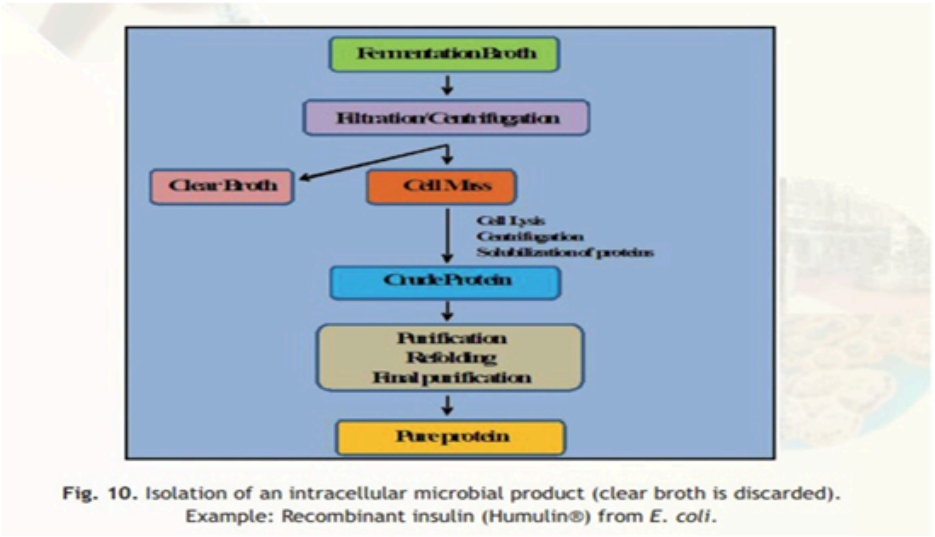
OR

(b) How is a continuous culture system maintained in microbial culture ? What are its advantages over Fed-batch culture ? 5

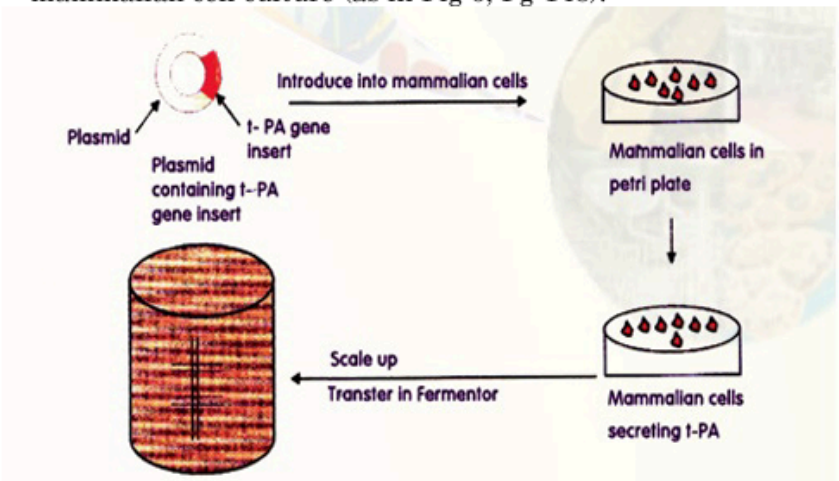
Answers of CBSE Board Question Paper 2025

MARKING SCHEME BIOTECHNOLOGY (045) SET-4 (Series ZXW4Y) Q.P. CODE 99 (2024-25)		
SECTION – A		
1	(B) T4 Bacteriophage	1
2	(A) Weight gain of an adult by consuming 1 g of food protein	1
3	(C) Four	1
4	(D) Alzheimer's disease	1
5	(B) Cereal grains	1
6	(A) M13 based vector	1
7	(C) SCID	1
8	(B) James Thomson	1
9	(A) GeneMark	1
10	(D) Whey protein concentrates	1
11	(C) Expression proteomics	1
12	(A) Therapy of early stage breast cancer	1
13	(A) Both Assertion (A) and Reason (R) are true and the Reason (R) is the correct explanation of the Assertion (A)	1
14	(B) Both Assertion(A) and Reason(R) are true but the Reason(R) is not the correct explanation of the Assertion(A).	1
15	(C) Assertion (A) is true, but Reason (R) is false.	1
16	(C) Assertion (A) is true, but Reason (R) is false.	1

17	(a) Principle: Mass spectrometery determines the molecular weight of chemical compounds by separating molecular ions according to their mass / charge (m/ z) ratio. **A**pplication: To obtain protein structural information such as peptide mass / amino acid sequence/ to identify type and location of amino acid modification within proteins/ to provide molecular weight of proteins. (any one) OR (b) The enzyme chymotrypsin is made up of a linear chain of 245 amino acids interrupted into three peptides. The protein folds into a globular structure and the three important amino acid residues His(57), Asp(102) and Ser(195) come close together in space which allows a 'charge relay system' to operate. The negatively charged aspartate (102) is able to form hydrogen bond with the adjacent histidine (57) partially borrowing a hydrogen ion from the latter. The His (57) makes good its partial hydrogen ion loss to Asp(102) by attracting a hydrogen ion from the adjacent Ser(195) through the His(57) residue making Ser(195) acidic in nature.	1+1=2 2
18	- **Any suitable example from Table 1, Pg 62** Table 1. Genome size and gene predictions between several organisms. **Organism** / **No. of chromosomes** / **Genome size in base pairs** / **The Number of Predicted genes** / **Part of the genome that encodes for protein** Bacteria *Escherichia coli* / 1 / 500,000 / 5000 / 90% Yeast *Saccharomyces cerevisiae* / 16 / 12,068,000 / 6340 / 70% Worm *Caenorhabditis elegans* / 6 / 100,000,000 / 19,000 / 27% Fly *Drosophila melanogaster* / 4 / 175,000,000 - 196,000,000 / 13,600 / 20% Weed *Arabidopsis thaliana* / 5 / 157,000,000 / 25,498 / 20% Human *Homo sapiens* / 23 / 3,000,000,000 / 20,000 - 25, 000 / < 5% -No correlation exists between the number of predicted genes, with the genome size and the number of chromosomes in an organism due to **overlapping genes, splice variants.**	1 ½ + ½ = 1

19	- Chronic mylogenous leukemia is caused due to 9 - 22 translocation in the chromosome resulting into shorter chromosome 22 (philadelphia chromosome) - Fluorescence In situ Hybridisation Technique/ Karyotype analysis (any one) **Alternative question for visually impaired in lieu of Q. 19.** The uses of the data provided in RefSeq database are: 1. Designing gene chips. 2. Describing the sequence features of the human genome.	1 1 1+1=2
20	-Steps for isolation of recombinant Insulin (Humulin) from *Escherichia coli* as depicted in Fig 10 Pg-100  **Fig. 10.** Isolation of an intracellular microbial product (clear broth is discarded). Example: Recombinant insulin (Humulin®) from *E. coli*.	2

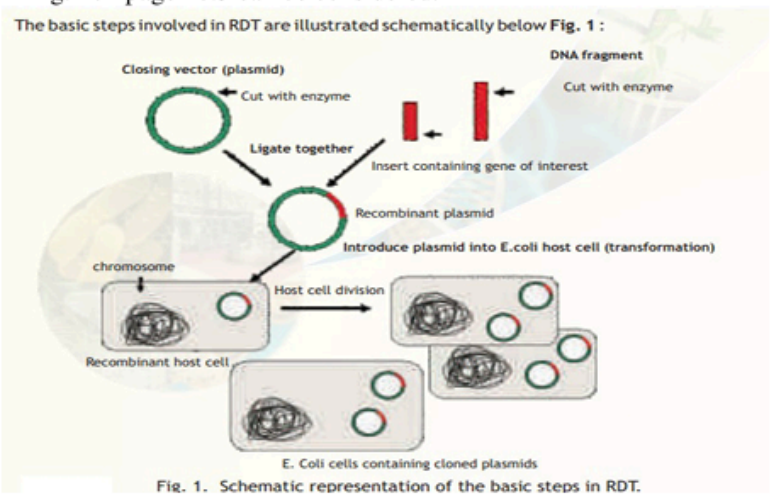
21	- Common cause of foaming in microbial culture medium is the presence of proteins in the culture medium. - Foaming denatures proteins and provides hindrance to free diffusion of oxygen in the medium.	1 ½ + ½ =1
	SECTION – C	
22	- Whey proteins result in the elevation of tripeptide glutathione (gamma-glutamyl cysteinyl glycine) in cells .Glutathione is a reducing compound which detoxifies xenobiotics and protects cellular components from the effect of oxygen intermediates and free radicals - Examples: Whey is used to treat various illnesses like jaundice, infected skin lesions, genito-urinary tract infections. (Any two examples.)	2 ½ + ½ =1
23	(a) Important features that were incorporated in each of the following vectors are : (i) COSMIDS : COS-sites of phage lambda, and features of plasmid (origin of replication, selectable marker, suitable restriction enzyme sites). (ii) Shuttle Vectors : Two types of origin of replication and selectable marker genes, one set which functions in the eukaryotic cells and another which functions in Escherichia coli. (iii) Expression Vectors : Signals necessary for transcription and translation of insert for expressing foreign protein. OR (b) - Blue –White selection method is based on the insertional inactivation of lac Z gene present on the vector pUC 19 . - The lac Z gene expresses the enzyme beta galactosidase which can cleave a colourless substrate called X-Gal into a blue coloured product - If Lac Z gene is inactivated due to the presence of the insert, then the enzyme is not expressed. - After a transformation experiment the E.coli host cells are plated on an ampicillin and X-Gal containing solid media plate - Colonies which appear blue are ampicillin resistant which have transformed cells but do not have insert. - Colonies which appear white are both ampicillin resistant and have the insert recombinant DNA.	1+1+1=3 ½ x 6 =3

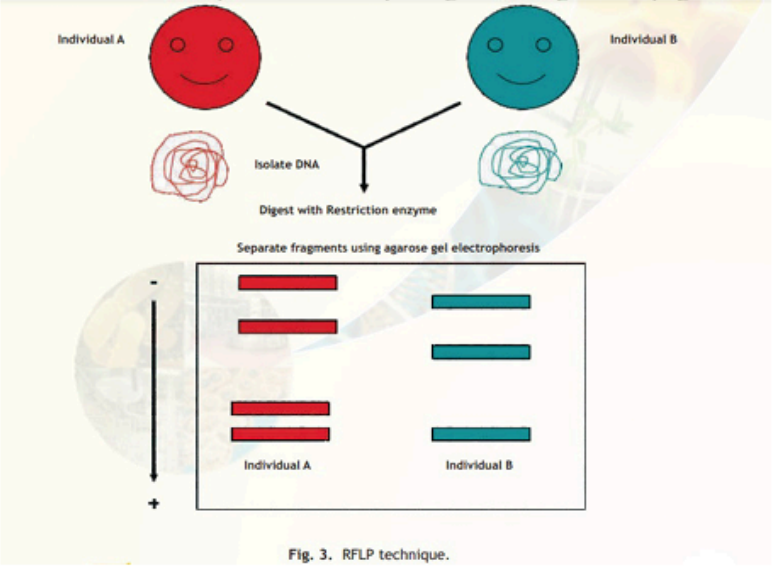
24	- Mode of action of tissue Plasminogen Activator (tPA): tPA converts plasminogen to plasmin, which dissolves blood clots. / tPA degrade - Plasminogen ------→ Plasmin--------→ Fibrin ----------→ Dissolution of blood clot (Inactive precursor enzyme)	1
	Schematic representation to show the method of production of tPA through mammalian cell culture (as in Fig 6, Pg-148).  Fig. 6. Production and mode of action of tPA.	2
	Alternative question for Visually Impaired in lieu of Q. 24. - Stem cells are cells that have the property of self-renewal through mitotic cell division and differentiation into a diverse range of specialized cell types.	1.
	-Two broad types of mammalian stem cells are –Adult stem cells and Embryonic stem cells. - Application of Adult stem cells- - Act as repair system for the body by (/) maintaining the normal turnover of regenerative organs (such as blood, skin or intestinal tissues). - Can be grown and transformed into specialized cells (such as muscles or nerves) through cell culture and (/) used in medical	½ + ½ =1

	therapies . - Can be used in medical conditions / where cells are either dead or injured or abnormal such as leukemia (cancerous blood cells), heart disease, heart attack (cardiac tissue damage), paralysis (spinal cord injury) , Alzheimer's , Parkinson's, Huntington's (dead brain cells), burns (damaged skin cells). **(Any one point)** (Any other relevant point can be considered) Application of Embryonic stem cells – -Can differentiate into cells of all types of specialized tissues. - Can be maintained in cell culture in the presence of irradiated fibroblast cells which can be reintegrated fully into embryogenesis if transferred. - Can be used to create Chimeric mice. - Can be used to create mouse models of human diseases. - Can be used to create mouse models with gene knock outs. **(Any one point)** (Any other relevant point can be considered)	½ ½
25	- Antibiotics are added in animal cell culture medium to control the growth of bacterial and fungal contaminants. - Two such antibiotics are Penicillin and Streptomycin	1 1+1=2
26	- It is very difficult to produce hybrids in case of interspecific and intergeneric crosses because of abnormal development of endosperm which causes premature death of hybrid embryo and leads to formation of sterile seeds. - Explanation of any one technique to obtain such novel hybrids: - Embryo rescue / Protoplast fusion to produce somatic hybrids and cybrids/ organelle transfer/organelle uptake .	1 2
27	- Engineering of Arabidopisis plant:- Three genes involved in PHB synthesis from *Alcaligenes eutrophus* were expressed exclusively in chloroplasts of Arabidopisis plant (to produce PHB globules), without affecting plant growth and development. - The drawback of producing PHB by fermentation using bacterium *Alcaligenes eutrophus* is high production cost.	2 1

28	- Red spots show genes expressed in high amounts in normal cells. - Green spots show genes expressed in high amounts in cancerous cells - Yellow spots show genes expressed approximately equally in both normal and cancerous cells .	1x3=3
	SECTION D	
29	(i) Only primers can be extended using single strand DNA template as a guide. (ii) The 3′OH group is present in dNTPs whereas 3′OH group is absent in ddNTPs (structures indicating correct labeling at 3' positions of dNTP and dd NTP can be considered). Function:- ddNTPs terminate the growing DNA chain where they are incorporated. (iii) Advantage:- Gels can be scanned by Laser / Danger of using radioisotopes is avoided / Single lane gel electrophoresis can be conducted instead of four lane gel. (Any one). **OR** (iii) DNA Polymerase	1 1 + 1 =2 1 1
30	(i) The culture of any piece of a part of a plant (explant) is known as explant culture. (ii) Auxin / Cytokinin / [Gibberellin] (any two) (iii) Plant cells without cell wall are known as " Protoplast" OR (iii) Micropropagation / Plant Regeneration / Preparation of single cell suspensions/ Preparation of protoplasts / Genetic transformation studies. (Any one)	1 1+1 =2 1 1

	SECTION E	
31	(a) The technique of peptide mapping used to compare normal haemoglobin with sickle cell haemoglobin:- 1, Pure Hb and scHb are taken separately into test tubes and are digested with the proteolytic enzyme trypsin. 2. Two separate strips of Whatman filter paper are spotted with Hb and scHb tryptic peptides and the peptides allowed to separate using the technique of paper electrophoresis at pH 2.0. 3. The paper strips are dried and chromatographed at right angles to the electrophoretic direction using a solvent system Butanol: Water:Acetic acid. 4, The chromatograms are dried and stained with a suitable visualisation reagent like Ninhydrin wherein peptide containing regions appear as orange yellow spots. 5. The peptide map for Hb and scHb are compared and the amino acid sequence of peptide differently placed in the scHb map is determined. OR (b) Any five protein based products as given below with one example of each:- 1. Blood products and vaccines. 2. Therapeutic antibodies and enzymes. 3. Therapeutic hormones and growth factors. 4. Regulatory factors. 5. Analytical application. 6. Industrial enzymes. 7. Functional non-catalytic proteins. 8. Nutraceutical proteins.	1x5 =5 (½+ ½) x5 =5

32	(a) The Basic steps of Recombinant DNA Technology are :- 1. Isolation of a DNA fragment containing a gene of interest that needs to be cloned (called as insert). 2. Generation of a recombinant DNA (rDNA) molecule by insertion of the DNA fragment into a carrier DNA molecule called vector (e.g. plasmid) that can self replicate within a host cell. 3. Transfer of the rDNA into an E. coli host cell (process called transformation). 4. Selection of only those host cells carrying the rDNA 5. Allowing recombinant cells to multiply thereby multiplying the rDNA Molecules / Flowchart with explanation of basic steps of Recombinant DNA Technology as given in fig.1 on page no.3 can be considered.  Fig. 1. Schematic representation of the basic steps in RDT.	1x5 =5

	OR (b) Procedure involved in Restriction Fragment Length Polymorphism (RFLP) Technique:- 1. Isolate test DNA samples 2. Digest test DNA samples with the same restriction enzyme 3. Separate the DNA fragments by agarose gel electrophoresis 4. Analysis of gel pattern. / Flowchart with explanation of steps as given in fig. 03 on page no.7 can be considered.  Fig. 3. RFLP technique. Application : To identify and relate individuals.	1x4 =4 1
33	(a) -The genomes contributed by both the culturable and the non-culturable variety of microbes together are termed as 'metagenome'. -Metagenomics approach:- The collective DNA is extracted from a sample of soil, water or any other environmental niche. The collective DNA is subjected to restriction digestion using restriction endonucleases. The DNA fragments obtained are cloned in suitable vectors. The clones are then screened for presence of a variety of molecules with improved characteristics.	1 3

	- Importance of metagenomics approach to study microorganisms is:- To cast a wider net on microbial resource present in the environment / to fish out genes of interest / to analyze the genomes of the microbes without culturing these in the laboratory / to study those microbes which are difficult to culture in the laboratory or have never been cultured in the laboratory as yet / analyze these microbes to see if they carry any genes which may be exploited for human use. OR	1
	(b) - In Continuous culture the growth medium is designed in such a way that one of the nutrients is in limited quantity. Thus, during the exponential growth just before the nutrient is fully exhausted, fresh medium containing the limited nutrient is added and this is repeated every time the limited nutrient is about to exhaust. -This system is also fitted with an overflow device so that the added volume displaces out an equal volume of culture from culture vessel. -In a chemostat, constant chemical environment is maintained whereas in a turbidostat constant cell concentration is maintained.	1x3=3
	Advantages of Continuous culture over Fed-batch culture :- - A steady state is achieved for an extended period of time. - Higher productivity. - Getting a continuous supply of microbial growth. - Easier control of constant growth conditions. - Continuous culture system can be maintained for a long period of time, (Any two advantages)	1+1=2

UNSOLVED BOARD QUESTION PAPER

BIOTECHNOLOGY (045) Class XII

Max.Marks:70 **Time allowed: 3 hours**

General Instructions:

i) All questions are compulsory.

ii) The question paper has five sections. All questions are compulsory.

iii) Section–A contains 12 Multiple choice questions and 4 Assertion-Reasoning based questions of 1 mark each; Section–B has 5 short answer questions of 2 marks each; Section –C has 7 short answer questions of 3 marks each; Section-D has two case-based question of 4 marks; Section-E has three long answer questions of 5 marks each.

iv) There is no overall choice. However, internal choices have been provided in some questions. A student has to attempt only one of the alternatives in such questions.

SECTION A **16x1=16**

1. The natural source of enzyme barnase and barstar, a system used to achieve male sterile plant is : *1*

(A) *Bacillus subtilis* *(C)* *Bacillus amyloliquefaciens*

(B) *Bartonella henselae* *(D)* *Barnesville coli*

2. Severe combined immunodeficiency disease is caused due to the absence of : *1*

(A) Adenosine diphosphate (C) Adenosine cyclase

(B) Adenosine deaminase (D) Guanidine nitrate

3. Single nucleotide polymorphisms usually occur in _____regions. *1*

(A) Coding (C) Regulatory

(B) Non-coding (D) Exonic

4. Artificial seeds are produced by encapsulating the somatic embryos at the _____stage in a protective coating. *1*

(A) Torpedo (C) Cotyledon

(B) Globular (D) Triangular

5. The peptide hormones and growth factors to promote healthy growth of animal cells *in vitro* are often derived from : *1*

(A) Phenol red (C) Blood serum

(B) Antibiotics (D) Amino acids

6. Identify the vector that infects *E. coli* cells containing F-plasmid and that has a single-stranded circular genome : *1*

(A) *Agrobacterium tumefaciens* (C) pBR322

(B) YEp (D) M13

7. An example of secondary metabolites produced by microbial cells include *1*

(A) Vitamins (C) Acids

(B) Alcohol (D) Antibiotics

8. When a transgene from a Genetically Modified crop escapes through pollen to a related plant species, it is known as _____. *1*

(A) Gene transfer (C) DNA contamination

(B) Gene pollution (D) Toxicity transfer

9. A protein ion with a molecular weight of 10,000 Daltons carried a charge of 5^+ and was subjected to mass spectrometric analysis. Calculate its mass to charge ratio. *1*

(A) 2001 (B) 2000

(C) 2501 (D) 5001

10. Embryonic stem cells derived from blastocyst stage of the embryo are _____in nature *1*

(A) Totipotent (B) Pluripotent

(C) Multipotent (D) Bipotent

11. An improved strain of *Penicillium*, capable of producing higher concentration of antibiotic penicillin is: *1*

(A) Penicillium notatum *(C) Penicillium eutrophus*

(B) Penicillium chrysogenum *(D) Penicillium cerevisiae*

12. __cultures can be maintained for a prolonged period of time by repeated sub-culturing. *1*

(A) Ovary (C) Callus

(B) Protoplast (D) Mass cell

For Questions number 13 to 16, two statements are given one labelled as Assertion (A) and the other labelled as Reason (R). Select the correct answer to these questions from the codes (A), (B), (C) and (D) as given below.

(A) Both Assertion (A) and Reason (R) are true and Reason (R) is the correct explanation of the Assertion (A).

(B) Both Assertion (A) and Reason (R) are true, but Reason (R) is ***not***

the correct explanation of the Assertion (A).

(C) Assertion (A) is true, but Reason (R) is false.

(D) Assertion (A) is false, but Reason (R) is true.

13. *Assertion (A) :* Some experts believe that there must be more than 30,000 genes in the human genome.

Reason (R) : Unreliability of in silico gene prediction is responsible for reporting a lesser number of genes in the human genome. *1*

14. *Assertion (A) :* The exact chemical composition of complex microbial growth media is known.

Reason (R) : Complex nutrient media is used when the specific growth requirement of a microorganism is unknown. *1*

15. *Assertion (A) :* The regulation of pH is essential for survival of mammalian cells.

Reason (R) : Animal cell cultures mostly make use of Bicarbonate – carbon dioxide buffering system to maintain pH. *1*

16. *Assertion (A) :* During plant tissue culture, the explants are treated with sodium hypochlorite.

Reason (R) : Sodium hypochlorite helps in acclimatization of the

regenerated plants.

SECTION B (2 Marks)

17. Illustrate steps to show the construction of a recombinant DNA molecule.

18. What are the advantages offered by creating a mouse model with gene knockout?

19. Name the technique that helps to study the entire protein profile from a given cell type. Briefly explain the principle of this method.

20. Write about any two strategies available to enhance the production of secondary metabolites in plant genetic engineering.

21. (a) Give any two drawbacks of animal cell culture in vitro.

OR

(b) (i) An oncologist is performing colony formation assay on tumour cells from a patient. What is he trying to determine?

(ii) Animal cells growing in culture show the property of contact inhibition. Relate this to what happens in an adult human body.

SECTION C (3 Marks)

22. (a) Compare the techniques of FISH with Microarray in terms of principle and applications.

OR

(b) Differentiate between Expression and Functional Proteomics.

23. Write the therapeutic use and the animal cell line employed in obtaining any ***three*** of the following protein pharmaceuticals: *3*

(A) Erythropoietin

(B) Herceptin

(C) Interleukin 2

(D) Tissue plasminogen activator

24. Explain the steps involved in PCR amplification method. 3

25. A researcher performed protein fingerprinting on hemoglobin from both normal and sickled red blood cells. Complete the flow-chart of the process by filling A, B, and C.
3

26. Discuss any three ways that can be employed to measure microbial cell growth.

27. What are zymogens? How is chymotrypsinogen different from chymotrypsin?
3

28. Give the names of any three genes that are used as selectable markers in recombinant DNA technology. Also mention the trait/protein they specify.

SECTION D (4 Marks)

29. Carefully read the below mentioned flow-chart and answer the questions that follow :

Flow-chart scheme for isolation of Protein A is as given below :

(a) Write whether Protein A is of intra or extra cellular origin. *1*

(b) Which step in the given purification scheme is metabolite specific? *1*

(c) Give the purification scheme for isolation of Humulin from *E. coli.* *2*

OR

(c)

Why is it advisable to use a lesser number of steps for downstream processing? *2*

30. Consider the following table and answer questions:

Given is a list of ingredients used for preparation of plant nutrient medium.

Plant Growth Media

Ingredients	Amount
NH_4NO_3	1650 mg/L
$CaCl_2$	440 mg/L
$MnSO_4$	22 mg/L
$FeSO_4$	27 mg/L
Glycine	2 mg/L
KNO_3	1900 mg/L
Sucrose	3 g/mL
Inositol	100 mg/L
EDTA	33 mg/L

(a) Which component in the given list is acting as the carbon source? *1*

(b) Which ingredient has been used to fulfill vitamin requirement? *1*

(c) Name two phytohormones which are generally added to prepare plant nutrient media. *2*

OR

Explain how the sterilization of the growth media is achieved in the laboratory.

SECTION E

31. (a)(i) Explain the reason for therapeutic use of whey proteins. *2*

(ii) Name any two diseases that have been treated with whey. *2*

(iii) Curd is advised to be administered with antibiotics. Why?1

(b) (i) Discuss the development of a novel protein. 3

(ii) Name any two properties that can be manipulated using protein engineering. 2

32. a) i) In Sanger's chain termination method, incorporation of ddNTP causes the growing DNA chains to terminate prematurely. Explain how.

ii) Briefly write the steps of Sanger's chain termination method.

OR

(b) (i) During DNA sequencing, why is the autoradiogram read from bottom to top to arrive at the original sequence? 2

(ii) Why is single tube DNA sequencing considered better and safer? 2

(iii) To perform DNA sequencing of a strand, we need to clone the sequence in a single-stranded form. Which vector will you prefer for this? 1

33. (a) (i) Name three database retrieval tools available from the NCBI. What all do they allow us to access? 3

(ii) What kind of information is available in UniProtKB and PDB databases? 2

OR

(b) (i) How is BLAST used to analyses sequence similarity? Explain. 3

(ii) Name the computer programmes that can perform gene prediction for bacterial and eukaryotic genomes. 2

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LINK OF MORE PAPERS

Solved Sample Question Paper (2024-25)

https://cbseacademic.nic.in/web_material/SQP/ClassXII_2024_25/Biotechnology-SQP.pdf

Marking scheme (2024-25)

https://cbseacademic.nic.in/web_material/SQP/ClassXII_2024_25/Biotechnology-MS.pdf

Chapter wise- list of scientists and their contributions

Chapter 1: Recombinant DNA Technology

Sl. No.	Scientists	Contribution
1	Paul Berg, Boyer, Chang, Cohen (1973)	First recombinant DNA molecule
2	W Arber, H Smith, and D Nathans	Discovery of Restriction enzymes
3	Mandel and Higa (1970)	Competent *E.coli* in cold calcium chloride solution
4	E M Southern	Southern Hybridization
5	Fred Sanger and Andrew Coulson	Dideoxy chain termination method of DNA sequencing
6	Maxam and Gilbert	Chemical degradation method of gene sequencing

Chapter 2: Protein Structure and Engineering

Sl. No.	Scientists	Contribution
1	Fred Sanger	Developed protein sequencing reagent FDNB and protein sequencing
2	Pehr Edman (1950)	Protein sequencing
3	Linus Pauling, GN Ramachandran, John Kendrew, Max Perutz	3-D structure of proteins by X-ray crystallography
4	V M Ingram (1957)	Protein Finger printing

Chapter 3: Genomics, Proteomics and Bioinformatics

Sl. No.	Scientists	Contribution
1	Thomas Roder (1986)	Coined Genomics
2	H Winkler (1920)	Coined Genome
3	J Craig Venter	ESTs to find genes
4	Zukerkandl and Pauling (1962)	Molecular evolution
5	Margret Dayhoff	PAM and protein evolution, Atlas of protein sequence
6	Rigby and Paul Berg	Nick translation

Chapter 5: Plant Tissue Culture and Applications

Sl. No.	Scientists	Contribution
1	Gottlieb Haberlandt	Father of PTC
2	Peter Beyer and Ingo Potrykus	Golden rice

Chapter 6: Animal Cell Culture and Applications

Sl. No.	Scientists	Contribution
1	George Gay	Established HeLa cell lines
2	Rous and Jones	Trypsin for subculture of adherent cells
3	Milstein and Kohler	Hybridoma Technology
4	Ernest McCulloch and James Till	Established stem cell research at University of Toronto
5	James Thomson (1998)	Isolate and grow human cells in culture

Chapter wise Abbreviations that you must know by now

Chapter 1: RECOMBINANT DNA TECHNOLOGY

1. RDT- Recombinant DNA Technology
2. rDNA- recombinant DNA
3. RM System- Restriction Modification System
4. RFLP- Restriction Fragment Length Polymorphism
5. GFP- Green Fluorescence Protein
6. MCS- Multiple Cloning Site
7. RF Form- Replicative Form
8. COS Site- Cohesive site
9. YAC- Yeast Artificial Chromosome
10. BAC- Bacterial Artificial Chromosome
11. TMV- Tobacco Mosaic Virus
12. pUC- plasmid University of California
13. PCR- Polymerase Chain Reaction
14. cDNA- complementary DNA
15. SV40- Simian Virus 40

Chapter 2: PROTEIN STRUCTURE AND ENGINEERING

16. RBC- Red Blood Cells
17. SCID- Severe Combined Immunodeficiency
18. ADA- Adenosine Deaminase
19. LMB- Laboratory of Molecular Biology
20. ACE-Acetylcholine esterase
21. SDS PAGE- Sodium dodecyl sulphate Polyacrylamide gel electrophoresis
22. IEF- Isoelectric focusing
23. pI- Isoelectric pH
24. GRAS- Generally regarded as safe
25. PEG- Polyethylene glycol
26. MS- Mass spectrometry

27. MALDI- Matrix assisted laser desorption/ ionization
28. INF- Interferon
29. t-PA- tissue Plasminogen activator
30. BCAA- Branched Chain Amino acid
31. PER-Protein Efficiency Ratio
32. BV- Biological Value

Chapter 3: GENOMICS, PROTEOMICS AND BIOINFORMATICS

33. EST- Expressed Sequence Tags
34. NCBI- National Centre for Biotechnology Information
35. SNP- Single Nucleotide Polymorphism
36. CFTR- Cystic Fibrosis Transmembrane Conductance Regulator
37. HTT- Huntingtin gene
38. FISH- Fluorescence in situ Hybridization
39. CML- Chronic Myelogenous Leukaemia
40. EMBL- European Molecular Biology Laboratory
41. EBI- European *Bioinformatics Institute*
42. PIR- Protein Information Resource
43. NBRF- National Biomedical Research Foundation
44. PDB- Protein Database
45. PALI- Phylogeny and Alignment
46. BLAST- Basic Local Alignment Search Tool

Chapter 4: MICROBIAL CELL CULTURE AND ITS APPLICATIONS

47. SCP- Single Cell Protein
48. NB- Nutrient Broth
49. LB- Lauria Broth
50. TSB- Tryptic Soy Broth
51. NA- Nutrient Agar
52. LA- Lauria Agar
53. TSA- Tryptic Soy Agar
54. ATCC- American Type Culture Collection
55. MTCC- Microbial Type Culture Collection and Gene Bank
56. NBAIM- *National Bureau of Agriculturally Important Microorganism*
57. PHB- Polyhydroxy butyrate
58. GMO- Genetically Modified Organism

Chapter 5: PLANT CELL CULTURE AND APPLICATIONS

59. MS- Murashige and Skoog medium
60. Ti Plasmid- Tumour inducing plasmid
61. Bt- Bacillus thuringiensis
62. PR Proteins- Pathogenesis Related proteins

63. LAF- Laminar Air Flow
64. HEPA- Hight Efficiency Particulate Air
65. EPO- Erythropoietin
66. IL2- Interleukin- 2
67. mAb- monoclonal Antibody
68. CHO- Chinese Hamster Ovary
69. r-HuEPO- Recombinant Human Erythropoietin
70. ES CELLS- Embryonic Stem Cell

e-Learning Resource Table

TOPIC	LINK
Unit V	**Protein and Gene Manipulation**
Restriction Digestion- VLAB	https://mbvi-au.vlabs.ac.in/molecular-biology-1/Restriction_Digestion/
Polyacrylamide Gel Electrophoresis-VLAB	https://mbvii-au.vlabs.ac.in/exp/polyacrylamide-gel-electrophoresis/
Extraction of DNA from Agarose gel-VLAB	https://mbvi-au.vlabs.ac.in/molecular-biology-1/Extraction_of_DNA_from_Agarose_gel/
Preparation of Competent Cell (Calcium Chloride Treatment)-VLAB	https://mbvi-au.vlabs.ac.in/molecular-biology-1/Preparation_of_%20Competent_Cell/
Transformation of the Host Cells-VLAB	https://mbvi-au.vlabs.ac.in/molecular-biology-1/Transformation_of_the_Host_Cells/
Polymerase Chain Reaction-NPTEL	Course Name : Proteomics and Genomics
Polymerase Chain Reaction (PCR)-VLAB	https://mbvii-au.vlabs.ac.in/exp/polymerase-chain-reaction/
Genetic Engineering: Theory and Application-NPTEL	Genetic Engineering: Theory and Application
Isoelectric Focusing (IEF)-NPTEL	Course Name : Proteomics and Genomics
Mass spectrometry based proteomics-NPTEL	Mass spectrometry based proteomics
Proteins and Gel-Based Proteomics-NPTEL	Proteins and Gel-Based Proteomics
NCBI	NCBI
Proteomics: Principles and Techniques-NPTEL	https://nptel.ac.in/courses/102101007
Pairwise Sequence Alignment using BLAST-VLAB	Pairwise Sequence Alignment using BLAST (Theory) : Bioinformatics Virtual Lab II : Biotechnology and Biomedical Engineering
Retrieving articles using PubMed-VLAB	Retrieving articles using PubMed (Theory) : Bioinformatics Virtual Lab I : Biotechnology and Biomedical Engineering
Retrieving sequence data from Entrez-VLAB	Retrieving sequence data from Entrez (Theory) : Bioinformatics Virtual Lab I : Biotechnology and Biomedical Engineering
Unit VI	**Cell Culture and Genetic Manipulation**
Cell Culture Technologies-NPTEL	Cell Culture Technologies

Cell culture-NPTEL	Course Name : Cell Biology
Basics of Plant Tissue Culture-VLAB	https://cbi-au.vlabs.ac.in/exp/plant-tissue-culture/
Maintenance of Mammalian Cell Lines-VLAB	https://cbii-au.vlabs.ac.in/exp/mammalian-cell-lines/index.html
Bacterial Growth Curve-VLAB	Bacterial Growth Curve