जैव प्रौद्योगिकी

BIOTECHNOLOGY

कक्षा/Class: XII

2024-25

विद्यार्थी सहायक सामग्री

Student Support Material

तत् त्वं पूषन् अपावृणु
केन्द्रीय विद्यालय संगठन

केन्द्रीय विद्यालय संगठन

Kendriya Vidyalaya Sangathan

संदेश

विद्यालयी शिक्षा में शैक्षिक उत्कृष्टता प्राप्त करना केन्द्रीय विद्यालय संगठन की सर्वोच्च वरीयता है। हमारे विद्यार्थी, शिक्षक एवं शैक्षिक नेतृत्व कर्ता निरंतर उन्नति हेतु प्रयासरत रहते हैं। राष्ट्रीय शिक्षा नीति 2020 के संदर्भ में योग्यता आधारित अधिगम एवं मूल्यांकन संबन्धित उद्देश्यों को प्राप्त करना तथा सीबीएसई के दिशा निर्देशों का पालन, वर्तमान में इस प्रयास को और भी चुनौतीपूर्ण बनाता है।

केन्द्रीय विद्यालय संगठन के पांचों **आंचलिक शिक्षा एवं प्रशिक्षण संस्थान** द्वारा संकलित यह 'विद्यार्थी सहायक सामाग्री' इसी दिशा में एक आवश्यक कदम है । यह सहायक सामग्री 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 सामाग्री-संकलन की विशेषज्ञता के लिए जानी जाती है और अन्य शिक्षण संस्थान भी इसका उपयोग परीक्षा संबंधी पठन सामग्री की तरह करते रहे हैं । शुभ-आशा एवं विश्वास है कि यह सहायक सामग्री विद्यार्थियों की सहयोगी बनकर सतत मार्गदर्शन करते हुए उन्हें सफलता के लक्ष्य तक पहुंचाएगी ।

शुभाकांक्षा सहित ।

निधि पांडे
आयुक्त, केन्द्रीय विद्यालय संगठन

STUDENT SUPPORT MATERIAL

ADVISOR

Mrs. Nidhi Pandey, IIS, Commissioner,
KVS (HQ), New Delhi

CO-ORDINATION TEAM AT KVS (HQ)

1. **Ms. Chandana Mandal, Joint Commissioner (Training)**
2. **Dr. P. Devakumar, Joint Commissioner (Academics)**
3. **Dr. Ritu Pallavi, Assistant Commissioner (Training)**

CONTRIBUTORS

1. **Mrs. Shahida Parveen, Director, ZIET MUMBAI**
2. **Mr. Lakshmi Narayanan, Principal, PM SHRI K.V Virudhunagar, CHENNAI Region**
3. **Mrs. Remya P. Iyer, PGT (Biotech.) K.V IIT, Guwahati , GUWAHATI Region**
4. **Ms. Nabaneeta Das , PGT (Biotech.) K.V IIT, Kharagpur, KOLKATA Region**
5. **Mr. Abhinav Goyal, PGT (Biotech.) PM SHRI K.V Khanapara, GUWAHATI Region**
6. **Mrs. Arpita Basu, PGT (Biotech.) PM SHRI K.V No.1 Saltlake, Kolkata KOLKATA Region**
7. **Ms. Ajanta Panda PGT (Biotech.) PM SHRI K.V No.1 Sambalpur, BHUBANESWAR Region**
8. **Mr. Anand K PGT (Biotech.) PM SHRI K.V Ganeshkhind, Pune, MUMBAI Region**

Special Acknowledgement

1. **Mrs. Sneha Nandekar,PGT(Biotech.) PM SHRI K.V No.2 Colaba, MUMBAI, Region**
2. **Mrs. Neha Gera Chawla , PGT (Biotech.) K.V INS Hamla, MUMBAI Region**
3. **Mrs. Babita Thukral, PGT (Biotech.) K.V IIT Powai, MUMBAI Region**

REVIEW TEAM

1. **Mr. T. Narayana Dhas TA Biology ZIET MUMBAI**
2. **Mr. S. N Ojha PGT (Biology) PM SHRI K.V No. 1 REWA, JABALPUR Region**

CLASS XII BIOTECHNOLOGY

INDEX

EXAMINATION TIPS

- Know the chapters to be covered and the weightage for each chapter.
- Know the Question paper pattern along with weightage for various types of questions (VSA, SA, Case-based, LA – Type)
- Learn the definition of various terms.
- Practice important diagrams by drawing & labelling
- Make your own notes in simple language in a way you can understand & remember.
- You can discuss / teach what you have learnt with/to the peer group. The more yoy share the more you remember.
- Have a time table for self-study at home More time is to be allotted for the subjects which require deep & concentrated study. Combination of tough and easy subjects in a day will keep you away from getting bored and tired.
- Write important formulae/ name of scientists & their contribution & display them in your study room in prominent places and make a habit to put a glance at them whenever possible.
- Try to study in early hour of the day as it leads to more retention of concepts.
- Fast and legible handwriting is essential for Exam. This can be mastered only when practiced throughout the year.
- Write as many dummy tests / exams as possible at home apart from the ones administered in the school
- Till the syllabus is completed, you can write and practice chapter-wise tests with a schedule of one subject in a day.
- Sample Question papers from CBSE site along with Student Support Material provided in the school will be of great help for self-administered tests/exams.
- You can practice 3-hr exam by randomly selecting previous years question papers from CBSE.
- You should know and restrict to the word-limit of VSA/SA/LA-Type questions.
- Try to write the known answer as fast as possible and save time for other answers to think, recall and write.
- Wherever required, show the steps especially while answering the questions as step-wise marking pattern is also followed by CBSE.

All the Best

CLASS XII (2024-25)

COURSE- STRUCTURE- (THEORY)

One Paper **Max. Marks 70+30**

Time: 3 hrs.

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

One paper **Time: 3 hrs.**

Total 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 theacademic 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 Text Book 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 Text Book 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)

2024-25 Class XII

Biotechnology (045)

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

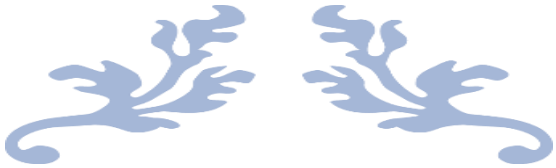
Competencies	
Demonstrate, Knowledge andUnderstanding	50%
Application of Knowledge / Concepts	30%
Analyse, Evaluate and Create	20%

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.



BIOTECHNOLOGY

CLASS XII

UNIT-5

PROTEIN AND GENE MANIPULATION

CHAPTER-5.1

RECOMBINANT DNA TECHNOLOGY

5.1.1. Recombinant DNA Technology- Introduction

The intrinsic capabilities of a cell are due to the genes contained by it.

Genes are made up of DNA (deoxyribonucleic acid) and they produce their phenotypic effects through transcription and translation (gene expression). Copies of DNA in the cell are produced through semi-conservative replication. The high fidelity of DNA replication ensures transmission of genes from parents to progeny without change. This is the reason for stability of genetically controlled (controlled by genes) phenotypes over generations. However, a low frequency (10^{-4} to 10^{-7} per gene per generation) of changes occur in genes naturally (spontaneous mutation); these mutations are the ultimate source of all the heritable variations observed in living forms.

The natural processes of gene transfer among organisms vary appreciably in their range and specificity. Generally, there are certain limits on movement of genes across taxonomic borders. However, during evolution, genes appear to have moved across great taxonomic distances; for example, many bacterial genes have been directly integrated into the human genome during the course of evolution.

One would like to reproduce such and even more distant gene transfers in a controlled manner and at a very high enough rate to be of practical application. This is achieved by the technique of recombinant DNA technology, which deals with the production of **recombinant DNA (the DNA molecule that is produced by joining together two or more DNA segments usually originating from different organisms)** for achieving one of the following three Objectives: -

1) To obtain a large number of copies of a specific DNA fragment/a gene of interest/a desired gene
2) To recover large quantities of the protein encoded by the gene of interest/desired gene
   ex : production of human insulin using *E. coli* as host
   production of hepatitis B vaccine using *yeast* as host
3) To integrate the gene of interest/desired gene into the genome of a target organism where it expresses itself leading to the development of a desired phenotype in the organism concerned
   ex : production of insect resistant cotton plants

❖ **Even for the latter two objectives, it is essential to first obtain a large number of copies of the concerned gene. In the second case, the protein product of the gene is the item of interest; it is isolated from the organism and purified. But in the third case, modified phenotype of the organism is the feature of interest, since this enhances, in some way, the usefulness of the organism.**

The pure multiple copies of desired gene can be obtained in the following two ways:-

1) Gene Cloning: The process of obtaining multiple copies of a gene inside a living organism by the advent of recombinant DNA technology.
2) Polymerase Chain Reaction: The process of obtaining multiple copies of a gene inside a tiny tube using some biochemical components in an automatic machine.

5.1.2. STEPS OF GENE CLONING

The entire procedure of gene cloning or recombinant DNA technology may be classified into the following steps:-

1) Isolation of the DNA fragment containing the gene of interest/desired gene that needs to be cloned, i.e., ***DNA insert***.

2) Generation of a recombinant DNA (rDNA) molecule by Insertion of the isolated gene in a suitable carrier DNA molecule, i.e., ***vector***, that is capable of autonomous replication/multiplication within a host cell.

3) Introduction of the recombinant DNA into a suitable organism /cell (usually *E. coli*) called ***host*** (process called transformation).

4) Selection of only those host cells carrying the rDNA and allowing them to multiply leading to the multiplication of rDNA molecules along with multiplication/expression of the gene of interest lying within the rDNA.

5) Where needed, transfer and expression of the gene into another target organism to obtain the desired protein/phenotype in the same.

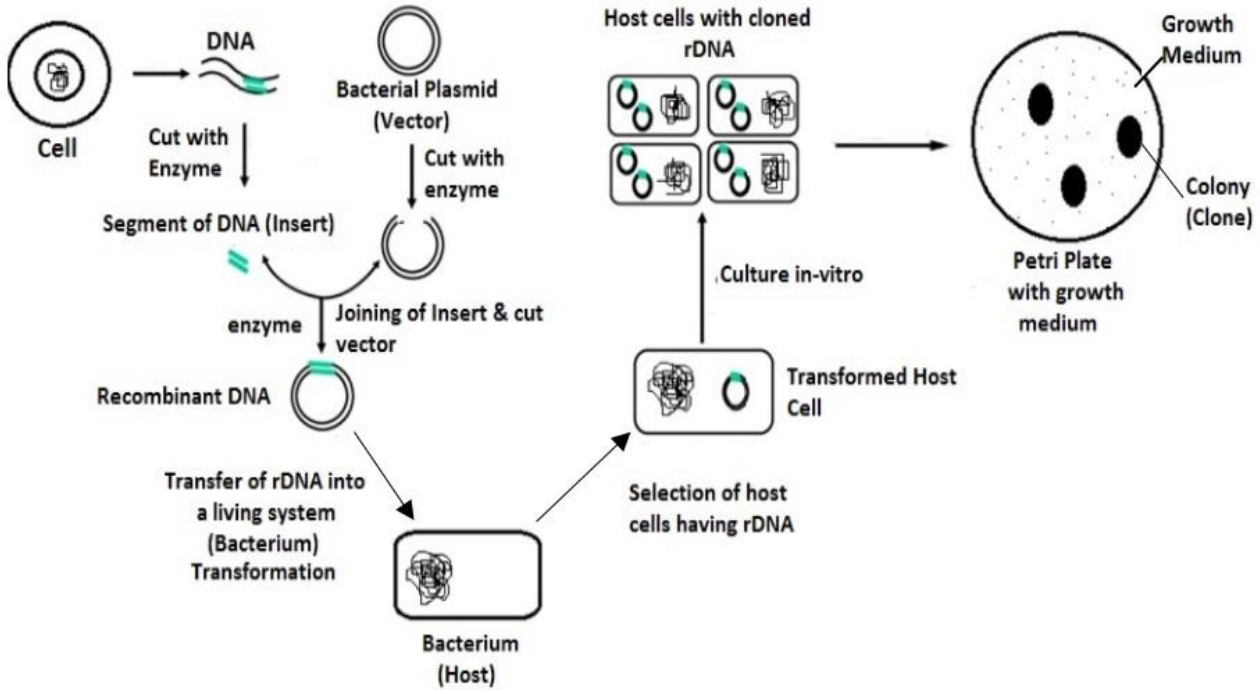


Fig 1: Basic Steps of Gene Cloning

5.1.3. TOOLS OF rDNA TECHNOLOGY

5.1.3.1. Restriction Enzymes: Molecular Scissors

Endonucleases are enzymes that produce internal cuts, called **cleavage,** in DNA molecules. (An **exonuclease** removes one nucleotide at a time from the end of a DNA molecule; it does not produce internal cuts in DNA.) A type of endonucleases which cleaves DNA only within or near those sites, which have specific base sequences, are known as **restriction endonucleases**, and the sites recognised by them are called **recognition sequences** or **recognition sites** or **restriction sites.** The recognition sequences are different and specific for the different restriction endonucleases/enzymes. **Restriction enzymes are essential tools for cutting DNA molecules at specific predetermined sites.**

Restriction enzymes exist in many bacteria where they function as a part of their defence mechanism called the **Restriction Modification** system.

(The presence of restriction enzymes in bacteria was postulated

by W. Arber during 1960s.)

Restriction-Modification system consists of two components:

1) The first component is a restriction enzyme that selectively recognises a specific DNA sequence and degrades any DNA molecule containing that sequence. The term **'restriction''** refers to the function of these enzymes in restricting the propagation of foreign DNA (e.g., bacteriophages) in a bacterium.

2) The second component is a modification enzyme that adds a methyl group to one or two bases usually within the sequence recognised by the restriction enzyme. Once a base in a DNA sequence is modified by the addition of a methyl group, the restriction enzymes fail to recognize and cleave that DNA. This is how a bacterium modifies and

therefore protects its own chromosomal DNA from cleavage by these restriction enzymes.

Different species of bacteria contain their own sets of restriction endonucleases and corresponding methylases.

5.1.3.1.1. Types of Restriction Endonucleases

There are following three distinct types of restriction endonucleases:-

1) **Type I restriction endonucleases** are complex endonucleases, and have recognition sequences of about 15 bp; they cleave the DNA about 1000 bp away from the 5'-end of the sequence "TCA" located within the recognition site, e.g., *Eco*K, *Eco*B, etc.

2) **Type II restriction endonucleases** are remarkably stable and induce cleavage either within or immediately outside their recognition sequences, which are symmetrical. They require Mg^{2+} ions as co-factor for inducing cleavage. Only Type II restriction endonucleases are used in recombinant DNA technology in view of their cleavage only at specific sites, e.g., *Eco*RI, *Hin*dII.

3) **Type III restriction endonucleases** are intermediate between Type I and Type II enzymes. They cleave DNA in the immediate vicinity of their recognition sites, e.g., *Eco*PI, *Eco*P15, *Hinf*III, etc. Type III enzymes recognise asymmetric target sites, and cleave the DNA duplex on one side of the recognition sequence upto 20 bp away.

****Type I and Type III restriction enzymes are not used in recombinant DNA technology.**

Table: Type II restriction enzymes & their restriction site

restriction enzyme	restriction site
BamH I	5' G|G A T C C 3' 3' C C T A G|G 5'
EcoR I	5' G|A A T T C 3' 3' C T T A A|G 5'
Hae III	5' G G|C C 3' 3' C C|G G 5'
Hha I	5' G|C G C 3' 3' C G C|G 5'
Hind III	5' A|A G C T T 3' 3' T T C G A|A 5'

5.1.3.1.2. Nomenclature

Abbreviation	Meaning	Description
E	*Escherichia*	genus
co	*coli*	species
R	RY13	strain
I	First	order of identification in the species

The nomenclature of restriction enzymes follows a general pattern : (1) The first letter of the name of genus in which a given enzyme is discovered is written in capital. (2) This is followed by the first two letters of species name of the organism. These three letters are generally written in italics, e.g., *Eco* from ***E****scherichia* ***co****li, Hin* from ***H****aemophilus* ***in****fluenzae, etc.* (3) Strain or type identification is depicted, e.g. *Eco*K (K- name of strain), *Eco*R (R-name of strain), etc. (4) When an organism produces more than one enzyme, they are identified by sequential roman numerals suggesting their order of discovery in that organism, e.g., the different enzymes produced by *H. influenzae* strain Rd are named *Hin*dII, *Hin*dIII, etc.

5.1.3.1.3. Recognition Sequences

The recognition sequences for type II restriction enzymes form **palindromes with rotational symmetry**. In a **palindrome,** the base sequence in the second half of a DNA strand is the mirror image of the sequence in its first half; consequently, the complementary DNA strand of a double helix also shows the same situation (Fig. 2). But in a **palindrome with rotational symmetry**, the base sequence in the first half of one strand of a DNA double helix is the mirror image of the second half of its complementary strand (Fig. 3). Thus in such palindromes, the base sequence in both the strands of a DNA duplex reads the same when read from the same end (either 5' or 3') of both the strands. Most of the Type II restriction endonucleases have recognition sites of 4, 5 or 6 bp (base pairs), which are predominantly GC- rich.

Fig. 2 A palindromic sequence. (a) Sequence in a single DNA strand. (b) Sequence in a DNA double helix. The arrow represents the axis of symmetry.

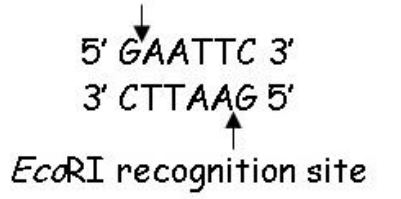


Fig. 3 A palindrome with rotational symmetry. The arrow represents the axis of symmetry.

5.1.3.1.4. Cleavage Patterns

Most type II restriction enzymes cleave the DNA molecules within their specific recognition sequences, but some produce cuts immediately outside the target/recognition sequence, e.g., *Nla*III, *Sau*3A, etc. These cuts are either (1) ***staggered*** or (2) ***even***, depending on the enzyme.

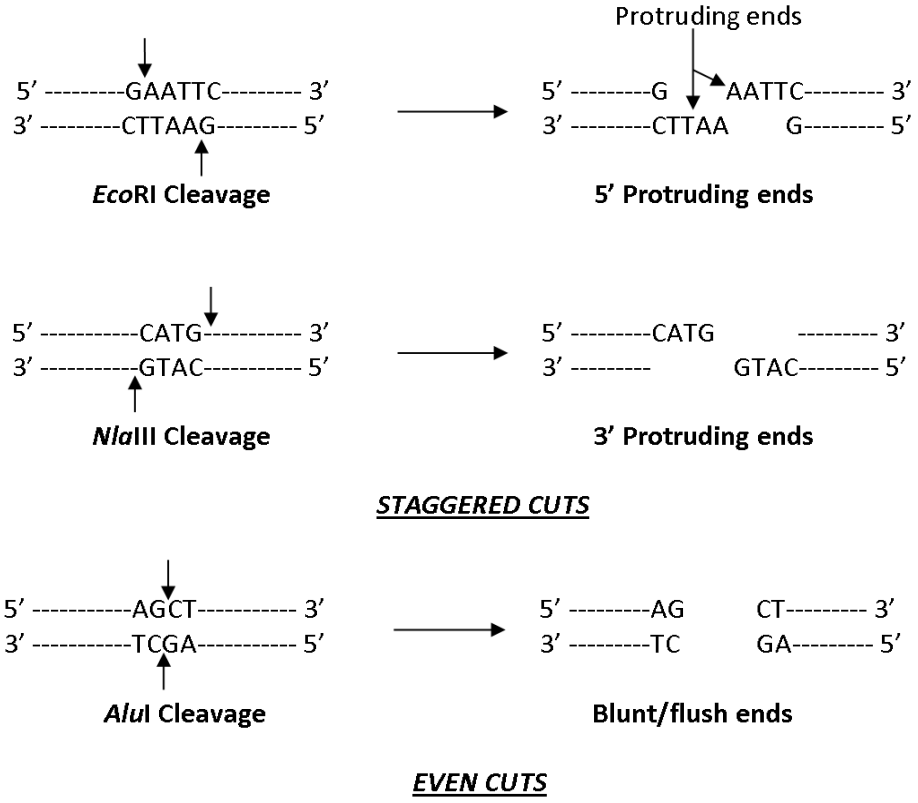
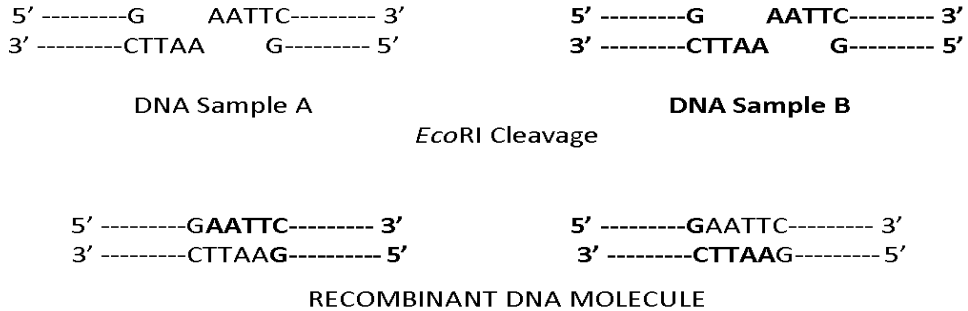


Fig. 4. DNA cleavage by restriction endonucleases. Staggered cuts: 5' protruding (*Eco*RI) and 3' (*Nla*III) protruding ends, and even cuts (*Alu*I) producing blunt/flush ends. The vertical arrows indicate the site of cut in DNA strand.

Most enzymes produce **staggered** cuts in which the two strands of a DNA double helix are cleaved at different locations; this



generates **protruding** (3' or 5') **ends** (Fig. 4), i.e., one strand of the double helix extends some bases beyond the other. Due to the palindromic (symmetrical) nature of the recognition sequences, the two protruding ends generated by such a cleavage by a given enzyme have complementary base sequence. As a result, they readily pair with each other under annealing conditions; such ends are called **cohesive** or **sticky ends. An important consequence of this fact is that when fragments generated by a single restriction enzyme from different DNAs are mixed, they join together due to their sticky ends** (Fig. 5)**. Therefore, this property of the restriction enzymes is of great value for the construction of recombinant DNAs.**

Fig.5. Two distinct samples (A and B) of DNA are cleaved with the same restriction enzyme (*Eco*RI). The fragments from sample A readily join with those from sample B due to their cohesive (or sticky = complementary) protruding ends.

Some restriction enzymes, on the other hand, cut both the strands of a DNA molecule at the same site so that the resulting termini or ends have **blunt** or **flush** ends in which the two strands end at the same point (Fig. 4). **The blunt cut ends can also be effectively utilised for construction of recombinant DNAs by using some special strategies.**

5.1.3.1.5. RFLP (Restriction Fragment Length Polymorphism)

RFLP is defined as differences among individuals in terms of the size of fragments generated by restriction enzyme digestion of genomic DNA. These differences are detected by agarose gel electrophoresis. RFLP denotes that a single restriction enzyme produces fragments of different lengths from restriction digestion of genomic DNA of different individuals of a species or different related species.

DNA isolated from an individual has a unique nucleotide sequence. Therefore, DNAs from different individuals rarely have exactly the same array of restriction sites for a given restriction enzyme. In other words, the locations of the restriction sites for a given restriction enzyme happens to be different in DNAs isolated

from different individuals. That's why, when DNA samples from different individuals are digested with

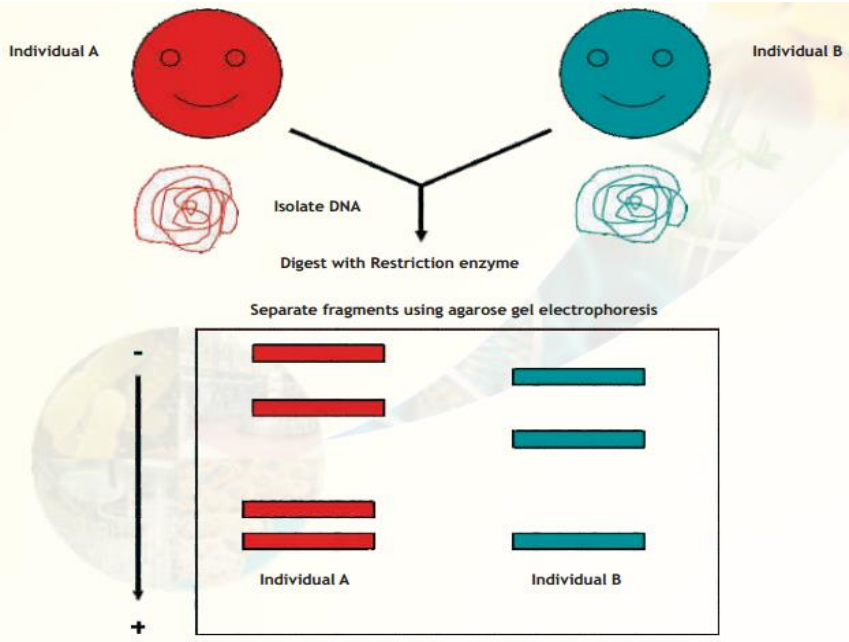


Fig 6: RFLP Technique

the same restriction enzyme, DNA fragments of varying lengths are generated, the phenomenon constitutes RFLP. When these fragments are resolved (according to size) on agarose gel using electrophoresis, pattern of DNA bands is obtained (RFLP pattern) which is unique for a particular person's DNA and reproducible with the same restriction enzyme again and again.

This technique can be used to distinguish between different DNA samples depending upon the differences in length of the DNA fragments generated by digestion with a given restriction enzyme.

A major application of RFLP is DNA finger printing which is frequently used in forensic science to identify and relate individuals.

5.1.3.2. Other Enzymes Used in Cloning

In addition to restriction enzymes, there are several other enzymes that play an important role in rDNA technology. Two of these are **DNA ligase** and **alkaline phosphatase**.

1) **DNA ligase:** This enzyme forms phosphodiester bond between adjacent nucleotides and covalently links two fragments of DNA. The reaction requires one of the fragments to have a 5' phosphate residue and the other a 3' hydroxyl group.

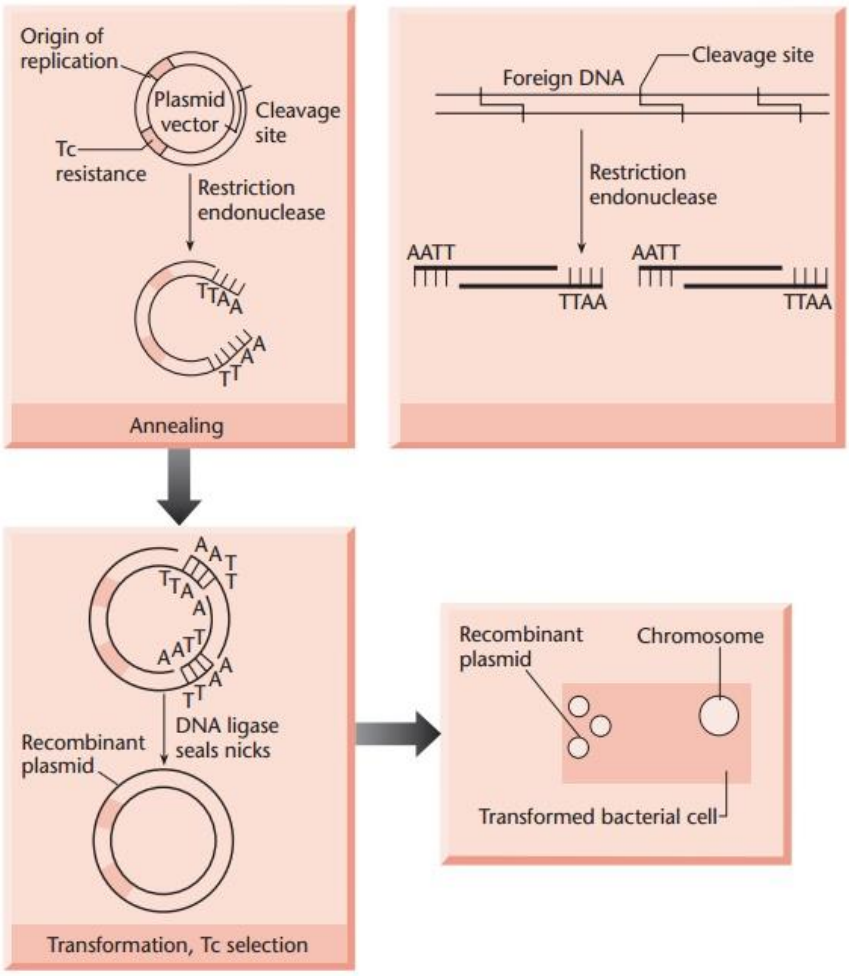


Fig 7. Use of DNA ligase to create a covalently closed recombinant DNA produced through association of termini generated by *Eco*RI.

In a previous section, it was indicated how two DNA fragments cut with *Eco*RI stick together; DNA ligase then joins covalently the annealed cohesive ends by forming a phosphodiester bond between the adjacent nucleotides (Fig. 7). DNA ligase isolated from bacteriophage T4 is frequently used for this purpose.

2) **Alkaline phosphatase:** Ligation requires the presence of 5' phosphate group. If some of the fragments are treated with alkaline phosphatase to remove their phosphate groups then these cannot ligate within themselves and are forced to ligate with other fragments containing 5' phosphate groups.

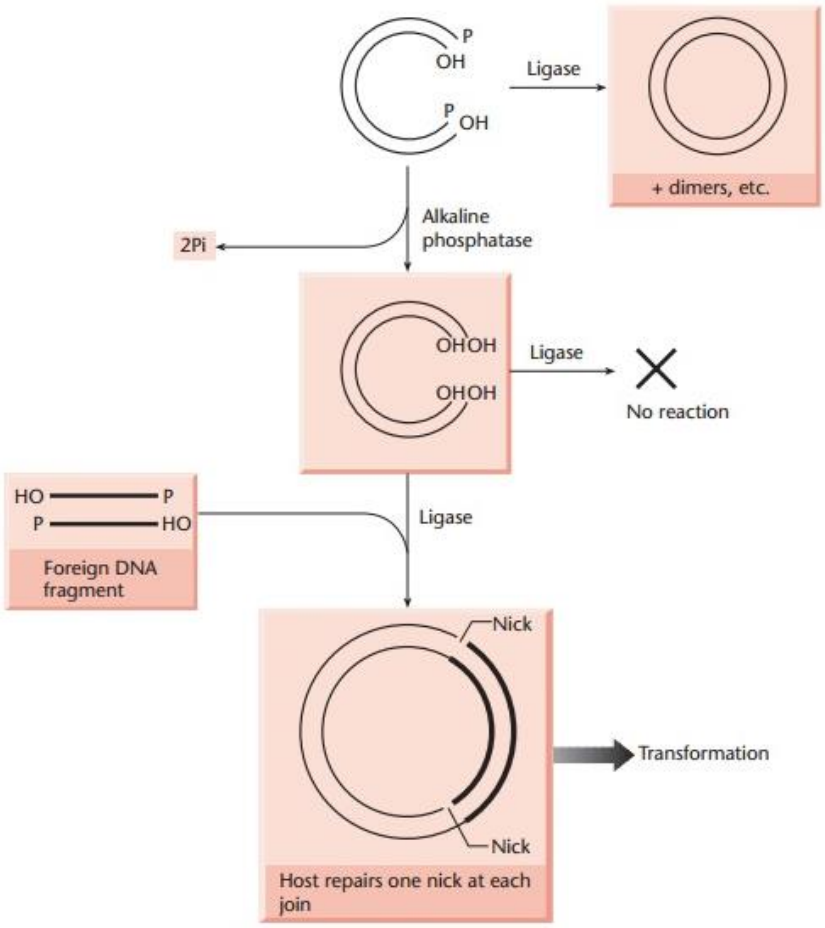


Fig 8. Application of alkaline phosphatase treatment to prevent unwanted recircularization of vector without insertion of foreign DNA/gene of interest.

One nick at each join remains unligated, but, after transformation of host bacteria, cellular repair mechanisms reconstitute the intact duplex (Fig. 8). Hence this is a useful strategy to prevent unwanted self-ligation of cut vector. An insert is ligated to the vector in generating rDNA as the vector is prevented from self ligation by treating it with alkaline phosphatase.

5.1.3.2. Vector- Vehicles for Cloning

A vector is a DNA molecule that has the ability to replicate autonomously in an appropriate host cell and into which the DNA

fragment to be cloned (called **DNA insert**) is integrated for cloning. Therefore, a vector must have an origin of DNA replication (denoted as **ori**) that functions in the chosen host. Vector also serves as a vehicle to carry a foreign DNA sequence/fragment into a host cell. Any extra chromosomal small genome, e.g., plasmid, phage or virus, may be used as a vector.

An ideal vector must have the following features:-

1) It should be able to replicate autonomously in the host cell. For that, it must have an *ori* sequence compatible with the chosen host.

2) A vector should be ideally less than 10 kb in size because large DNA molecules are broken during purification procedures and also impose difficulties during various manipulations required for gene cloning.

3) The vector should have suitable marker genes that allow easy detection and/or selection of the host cells transformed with recombinant DNA from among the cells transformed with unaltered vector and non-transformed cells.

4) A vector should contain unique restriction site for at least one restriction enzyme which can be used for cutting and introducing a DNA insert into vector. Most of the commonly used vectors have several unique restriction sites for multiple no. of restriction enzymes and these restriction sites are clustered at a site that is

known as **multiple cloning site (MCS)** or **polylinker**. A Polylinker provides flexibility in the choice of restriction enzyme(s) that can be used for cloning.

5.1.3.2.1. Plasmid Vectors

1) *p*BR322: The name *p*BR denotes the following: *p* signifies plasmid, B is from Boliver, and R is from Rodriguez; thus, B and R represent the initials of the scientists who developed *p*BR322. The numeral '322' distinguishes this plasmid from the other plasmids developed in the same laboratory, e.g., *p*BR325, *p*BR327, *p*BR328, etc. *p*BR322 is one of the most popular and most widely

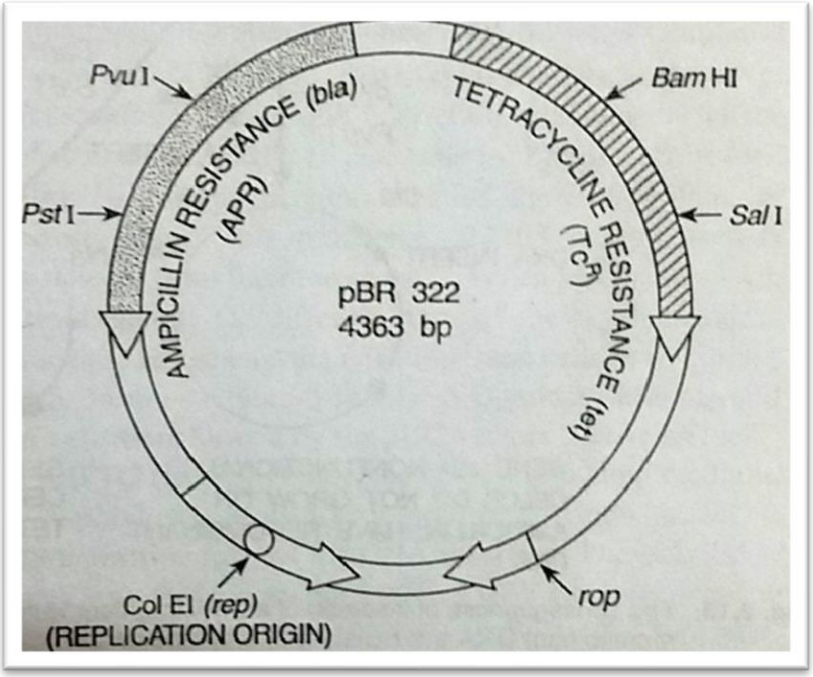


Fig. 9. Structural features of *p*BR322

used plasmid vector of 4363 bp. It has the replication module of *E. coli* plasmid ColE1. This replication module has been incorporated into many other plasmid vectors since it permits plasmid amplification/replication when protein synthesis is inhibited by amino acid starvation or treatment with chloramphenicol; as a result, under such conditions, each cell accumulates several

thousand (upto 3000) copies of the plasmid so that one litre of bacterial culture easily yields a milligram of plasmid DNA. It has two selectable markers (tetracycline, *tet*^{r} and ampicillin, *amp*^{r}, resistance genes) and only single or unique restriction/recognition sites for 12 different restriction enzymes (*Pst*I, *Pvu*I, located within the *amp*^{r} gene, and *Bam*HI, *Sal*I, etc. within *tet*^{r} gene). The presence of restriction sites within the markers *tet*^{r} and *amp*^{r} permits an easy selection of host cells transformed with the recombinant *p*BR322. ***rop*** gene (repressor of primer) regulates and reduces the plasmid copy number.

Insertional Inactivation

Insertion of DNA fragment into this plasmid using restriction enzyme *Pst*I or *Pvu*I, places the DNA insert within the gene *amp*^{r} and thus makes it non-functional. Bacterial cells containing such a recombinant *p*BR322 will be unable to grow in the presence of ampicillin, but will grow in the presence of tetracycline. Similarly, when restriction enzyme *Bam*HI or *Sal*I is used , the DNA insert is placed within the gene *tet*^{r} making it non-functional. The inactivation of a gene by insertion of a DNA sequence/segment (in this case the DNA insert) within it is known as **insertional inactivation**. Bacterial cells possessing such a recombinant *p*BR322 will, therefore, grow on ampicillin but not on tetracycline. The feature of insertional inactivation allows an easy selection of a single bacterial cell having recombinant *p*BR322 from among 10^8 other types of cells.

Identification and Selection of Recombinants:-

Transformed *E. coli* cells are first plated on an agar medium containing that antibiotic the resistance gene for which is intact, and the DNA fragment is not inserted, within this gene. Therefore, bacterial cells having the recombinant DNA are expected to be resistant to this antibiotic. This step eliminates non-transformed bacterial cells; the resulting bacterial colonies will posses either recombinant or unaltered *p*BR322. The colonies so obtained are then replica-plated on agar plates containing the other antibiotic (within

the gene for which the DNA insert is placed); all the colonies that develop on this plate will contain the unaltered *p*BR322. Therefore, the antibiotic sensitive colonies are identified and recovered from the master plate; these colonies will have recombinant DNA. This entire process may take 1-2 days.

2) *p*UC19: *p*UC series vectors are derivatives of *p*BR322 and are much smaller (around 2.7 kb). *p*UC19 has the following parts

(Fig. 10):

1) Ampicillin resistance gene (*bla*); marker gene
2) ColE1 origin of replication (*ori*)
3) A part of the *E. coli* gene *lacZ* ; this part of *lacZ* is denoted by *lacZ'* or *lacZa* , and encodes the N-terminal fragment, called the α fragment, of β-galactosidase, the enzyme that hydrolyses lactose. This enzyme can also hydrolyse X-gal (colourless substrate) converting it into a blue coloured product/dye.
4) A polylinker sequence or multiple cloning site (MCS) located within the *lacZa* provides several unique restriction sites for DNA insertion. The polylinker sequence is created in such a way that it does not interfere with *lacZa* expression by itself, but when a DNA insert is placed within it *lacZa* expression is prevented so that the *E. coli* cells possessing recombinant *p*UC19 are β-galactosidase deficient.

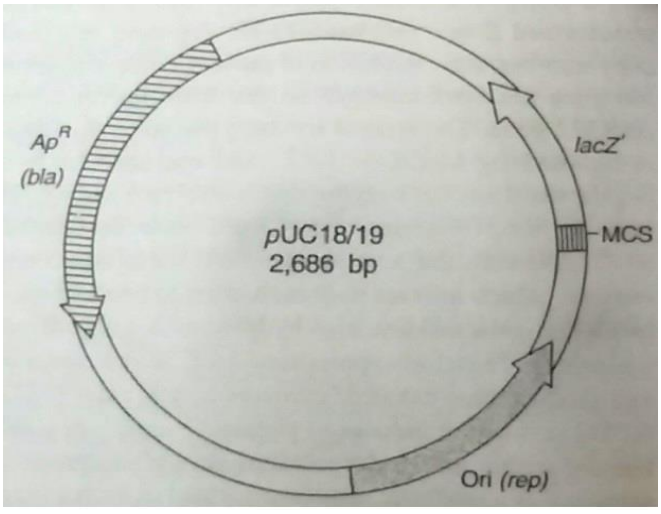
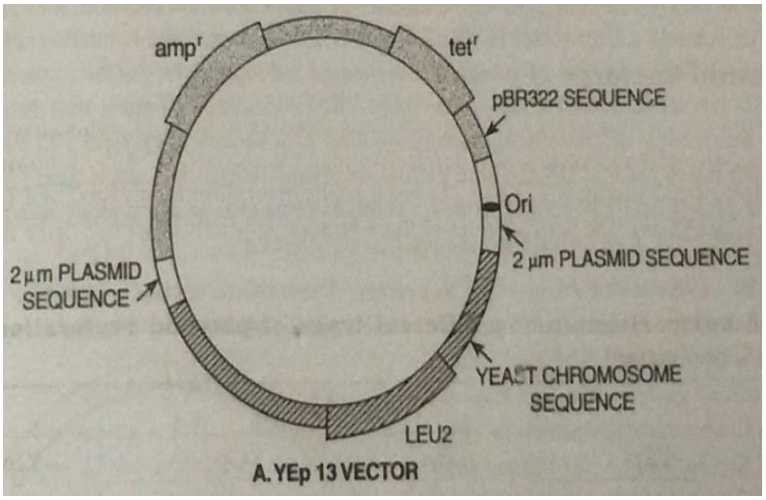


Fig 10- Structure of *p*UC19 vector

Identification and Selection of Recombinants:-The method of screening for the presence of recombinant *p*UC19 is referred to as blue- white selection. This method is based upon the insertional inactivation of the lac Z' gene present on the vector. This gene expresses the enzyme beta-galactosidase whose activity can cleave a colourless substrate called X-Gal into a blue coloured product. If the lac Z' gene is inactivated due to the presence of the insert then the enzyme is not expressed. Hence if after a transformation experiment the *E. coli* host cells are plated on an ampicillin and X-Gal containing solid media plate then colonies which appear blue are those which have transformed cells (antibiotic resistant) but do not have the insert (express active enzyme). Colonies which appear white are both ampicillin resistant and have the insert, i.e., recombinant DNA and thus are the cells to be used for future experiments.

3) YE*p*13- This vector contains the entire *p*BR322 sequence plus the origin of replication from 2 µm plasmid (a 6,318 bp long plasmid found in some strains of yeast) and *LEU2* chromosomal gene from yeast which encodes an enzyme involved in leucine biosynthesis. These vectors contain several unique restriction sites for insertion of DNA segments (Fig. 11). *LEU2* functions as a selectable marker in yeast strains lacking in function of this gene, viz., *LEU2(-)* strains of yeast. Cells of *LEU2(-)* strains will be able to grow on the minimal medium

Fig 11. YEp13 Vector



(medium lacking leucine) only when they will be transformed by YEp13 vector.

This vector has modules compatible with both bacteria (prokaryote) and

yeast (eukaryote) so it can function in both kinds of host. Such a vector which can function in two types of hosts is known as shuttle vector.

5.1.3.2.2. Phage Based Vectors

Bacteriophages are viruses that infect bacterial cells by injecting their DNA into them and consequently take over the machinery of the bacterial cells to multiply themselves. The injected DNA hence is selectively replicated and expressed in the host bacterial cell resulting in a number of phages which eventually extrude out of the cell (lytic pathway) and infect neighbouring cells.

This ability to transfer DNA from the phage genome to specific bacterial hosts during the process of viral infection gave scientists the idea that specifically designed phage based vectors would be useful tools for gene cloning experiments.

1) **Lambda Phage as Vector-**Bacteriophage lambda has a double stranded, linear DNA genome containing 48,514 bp, in which 12 bases on each end are unpaired but complementary . These ends therefore are sticky and are called cohesive or cos sites and are important for packaging DNA into phage heads. An important feature of the lambda genome is that a large fragment in the central region of its genome is not essential for lytic infection of *E. coli* cells. Therefore, vectors have been designed such that this region can be replaced by foreign insert DNA. These phage based vectors allow cloning of DNA
fragments up to 23 kb in size.

2) **M13 Phage as Vector-** M13 is a filamentous phage which infects *E. coli* having a pilus (protrusion) which is selectively
present in cells containing a F plasmid (called F^+ cells). The genome of the M13 phage is a single stranded, circular DNA of 6407 bp. Foreign DNA can be inserted into it without disrupting

any of the essential genes. In the life cycle of the phage following infection of the host *E.coli* cell the single stranded DNA is converted to a double-stranded molecule which is referred to as the Replicative Form (RF). The RF replicates until there are about 100 copies in the cell. At this point, DNA replication becomes asymmetric and single stranded copies of the genome are produced and extruded from the cell packaged with protein as M13 particles.

The major advantage of developing vectors based on M13 is that genes cloned into M13 based vectors can be obtained in the form of single stranded DNA.

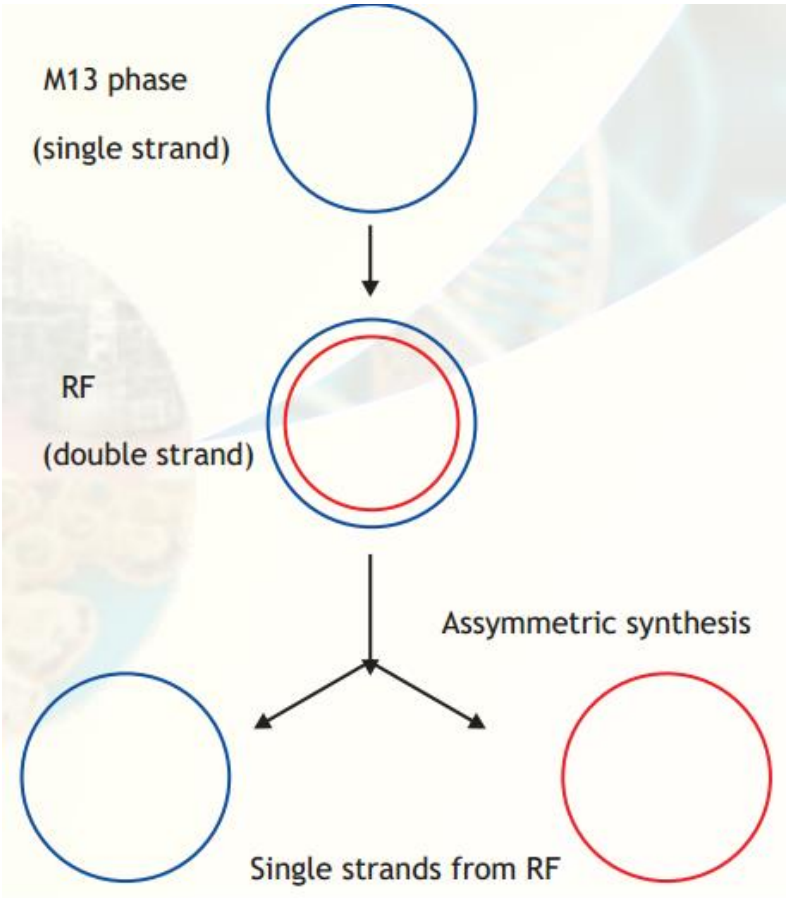


Fig 12. Life Cycle of M13 Phage

5.1.3.2.3. Cosmids

Cosmids have been constructed by combining certain features of plasmid and the 'cos' sites of phage lambda.

The simplest cosmid vector contains a plasmid, origin of replication, a selectable marker, suitable restriction enzyme sites and the lambda cos site. Cosmids can be used to clone DNA fragments up to 45 kbp in size.

5.1.3.2.4. YAC Vectors

Used to clone DNA fragments of more than 1 Mb in size. Therefore, they are useful in cloning larger DNA fragments as required in mapping genomes such as in the Human Genome Project.

These vectors contain a telomeric sequence, the centromere and an autonomously replicating sequence, features required to replicate linear chromosomes in yeast cells.

These vectors also contain suitable restriction sites to clone foreign DNA as well as genes to be used as selectable markers.

YAC is a kind of **shuttle vector** of *E. coli* and Yeast.

5.1.3.2.5. BAC Vectors

BACs or Bacterial Artificial Chromosomes are vectors based on the natural, extrachromosomal plasmid from *E. coli* - the fertility or F plasmid.

A BAC vector contains genes for replication and maintenance of the F plasmid, a selectable marker and cloning sites.

These vectors can accommodate inserts up to 500 kb and are used in genome sequencing projects

5.1.3.2.6. Animal and Plant Viral Vectors

A vector based on Simian Virus 40 (SV40) was used in the first cloning experiment involving mammalian cells.

A number of vectors based on other type of viruses like Adenoviruses and Papillomavirus have been used to clone genes in mammals.

At present, retroviral vectors are popular for cloning genes in mammalian cells. In case of plants, viruses like Cauliflower Mosaic Virus, Tobacco Mosaic Virus and Gemini viruses have been used with limited success.

Vector Type	Insert size (kb)
Plasmid	0.5-8
Bacteriophage lambda	9-23
Cosmid	30-40
BAC	50-500
YAC	250-1000

Table-
Common Cloning Vectors with their cloning capacity

5.1.3.3. Host Cells

During gene cloning experiment, after generation of r-DNA (vector combined with the gene of interest), the next step is to transfer/introduce this r-DNA into a living system or organism into which the r-DNA can replicate, that living organism is called as **host.** Many types of host cells including *E. coli*, yeast, animal and plant cells are available for gene cloning and the type of host cell used depends on the aim of the cloning experiment.

Properties of a Good Host

1) should be easy to transform
2) should support the replication of recombinant DNA
3) should be free from elements that interfere with the replication of r-DNA
4) lack active restriction enzymes, e.g., *E. coli* K12 substrain HB101
5) should not have methylases since these enzymes would methylate the replicated recombinant DNA which, as a result, would become resistant to useful restriction enzymes.

Bacteria are the hosts of choice for gene cloning. Among them, *E. coli* has become the most widely used host bacterium in gene cloning experiments because:

(1) its genetic makeup has been extensively studied
(2) it is easy to handle and grow
(3) can accept a wide range of vectors, some natural, some constructed
(4) has been extensively studied for safety
(5) under optimal conditions, the cells divide every 20 minutes making it possible to obtain large amount of cloned gene and if appropriate signals are incorporated (control elements) into the vector, large amount of recombinant protein (coded by the cloned gene) can also be obtained in a short period of time from *E. coli* cultures grown in fermenter (large culture vessel).

The *E. coli* strain K12 is the most commonly used bacterial host in gene cloning experiments: it has several substrains, e.g., C600, RR1, HB101, etc., each of which has some specific features important in cloning.

For the expression of eukaryotic proteins, eukaryotic host cells are often preferred because of the following two reasons:

a) Eukaryotic genes contain introns (non coding regions) present within their coding regions (split genes). These introns must be removed from primary transcripts for proper expression of the eukaryotic genes, but prokaryotes do not have the machinery needed for their removal from primary transcript.

(When eukaryotic genes are isolated as c-DNA, they are intron-free and suitable for expression in prokaryotes).

b) Eukaryotic proteins require proper folding and post translational modifications, such as glycosylation, to be functionally active, which is not possible in prokaryotic cells thereby necessitating the use of only eukaryotic host cells.

Yeast cells have been used extensively for functional expression of eukaryotic genes because of several features:

a) Yeasts are simplest eukaryotic organisms (unicellular and, like *E. coli*, have been extensively characterised genetically.

b) Easy to grow and manipulate.

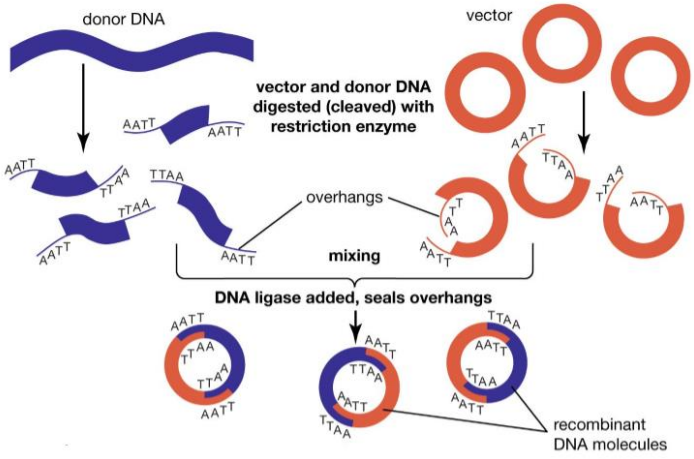
c) Large amount of cloned genes or recombinant proteins can be obtained from yeast cultures grown in fermenters (large culture vessels).

Plant and animal cells may also be used as host in r-DNA experiments and cells can be cultured in-vitro or can be induced and manipulated to form whole organisms (creation of transgenic plants and animals).

5.1.4. Making rDNA

1) To isolate the vector and the fragment containing the gene to be cloned using the same restriction enzyme.

2) To treat the cut vector with alkaline phosphatase enzyme



so that later in the ligation step the vector does not self-ligate.

3) The cut vector and DNA fragment are mixed in a suitable ratio and then ligated with the enzyme DNA ligase to yield a recombinant vector containing insert.

5.1.5. Introduction of rDNA into Host

- **Transformation-** In this procedure, bacterial cells take up DNA from the surrounding environment. Many host cell organisms such as *E. coli*, yeast and mammalian cells do not readily take up foreign DNA and have to be chemically treated to become competent to do so.
- **Transfection-** A method to transfer rDNA into host cells involves mixing the foreign DNA with charged substances like calcium phosphate, cationic liposomes or DEAE dextran and overlaying on recipient host cells.
- **Electroporation-** An electric current is used to create transient microscopic pores in the recipient host cell membrane allowing rDNA to enter.
- **Microinjection-** In the procedure of microinjection, foreign DNA is directly injected into recipient cells using a fine microsyringe under a phase contrast microscope to aid vision.
- **Biolistics-** Microscopic particles of gold or tungsten are coated with the DNA of interest and bombarded onto cells with a device much like a particle gun.
- Another method of introducing foreign genes is by the **natural genetic engineer *Agrobacterium tumefaciens*.**

5.1.6. Polymerase Chain Reaction

The polymerase chain reaction technique, developed by Kary Mullis in 1985, is extremely powerful. It generates microgram (µg) quantities of DNA copies (upto billion copies) of the desired DNA

segment, present even as a single copy in the initial preparation, in a matter of few hours.

The process of PCR consists of a series of reaction/amplification cycles and it is carried out *in vitro*. It utilizes the following : **(1)** a DNA preparation containing the desired DNA segment to be amplified (target sequence), **(2)** two oligonucleotide primers (about 20 bases long) specific, i.e., complementary, to the two 3'-borders (the sequences present at the 3'-ends of the two strands) of the desired segment, **(3)** the four deoxynucleoside triphosphates, *viz.,* dTTP (deoxythymidine triphosphate), dCTP (deoxycytidine triphosphate), dATP (deoxyadenosine triphosphate) and dGTP (deoxyguanosine triphosphate), and **(4)** a heat stable DNA polymerase, *e.g., Taq* polymerase (isolated from the bacterium *Thermus aquaticus*).

Procedure of PCR

At the start of PCR, the DNA from which a segment is to be amplified, an excess of the two primer molecules, the four deoxynucleoside triphosphates and the DNA polymerase are mixed together to form a reaction mixture that has appropriate quantities of Mg^{2+}.

A single PCR amplification cycle involves the following three basic steps (Fig. 13):

a) Denaturation

The reaction mixture is first heated to a temperature between 90-98ºC (commonly 94ºC) that ensures DNA denaturation (separation of both of the strands of DNA). This is the denaturation step. The duration of this step in the first cycle of PCR is usually 2 minutes at 94ºC.

b) Annealing

The mixture is now cooled to a temperature (generally 40-60ºC) that permits annealing of the primer to the complementary sequences in

the DNA; these sequences are located at the 3'-ends of the two strands of the desired segment. This step is called annealing. The duration of annealing step is usually 1 minute during the first as well as the subsequent amplification cycles of PCR. Since the primer concentration is kept very high relative to that of the template DNA, primer-template hybrid formation is greatly favoured over re-annealing of both the template strands.

c) Primer Extension/Polymerisation

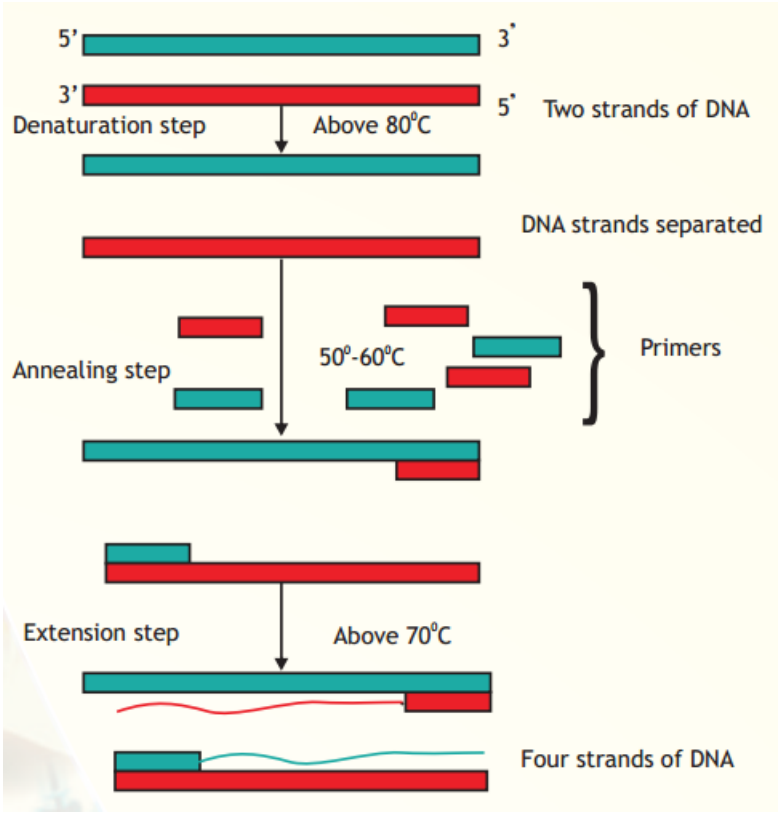
The temperature is now so adjusted that the DNA polymerase synthesizes the complementary strands by utilizing 3'-OH of the primers; this reaction is the same as that occurs *in vivo* during replication of the leading strand of a DNA duplex. The primers are extended towards each other so that the DNA segment lying between the two primers is copied; this is ensured by employing primers complementary to the 3'-ends of the segment to be amplified. The duration of primer extension is usually 2 minutes at 72ºC.

The completion of the extension step completes the first amplification cycle of PCR. Each cycle of PCR ordinarily takes 4-5 minutes. To begin the second cycle, the DNA is again heated to convert all the newly synthesized DNA into single strands, each of which can now serve as a template for synthesis of more new DNA. Thus, the extension products of one cycle can serve as a template for subsequent cycles and each cycle doubles the amount of DNA from previous cycle. As a result, from a single template DNA molecule, it

is possible to generate 2^n molecules after n number of cycles. Usually, 20-30 cycles are carried out in most PCR experiments. In case of automated PCR machines, called **thermal cyclers,** the researcher has to only specify the number and duration of cycles, etc. after placing the complete reaction mixture for incubation, and the machine performs the entire programme of operations precisely.

template DNA molecule, it is possible to generate 2^n molecules after n number of cycles. Usually, 20-30 cycles are carried out in most PCR experiments. In case of automated PCR machines, called **thermal cyclers,** the researcher has to only specify the number and duration of cycles, etc. after placing the complete reaction mixture for incubation, and the machine performs the entire programme of operations precisely.

Fig. 13. A schematic representation of the three steps performed during each reaction cycle of PCR



Applications of PCR

a) PCR can be used to detect the presence of a gene transferred into an organism (transgene) by using the 3'-end sequences of the transgene for amplification of DNA from the transgenic organism. Amplification will occur only when the transgene is present in the organism.

b) To generate abundant amount of DNA for analysis in the DNA fingerprinting technique used in forensic science to link a suspect's
DNA to the DNA recovered at a crime scene.

c) PCR can be used for detection of gene sequences of pathogens in biological samples to detect the infection by a pathogen.

d) PCR is used to detect the presence of a specific mutation that is responsible for causing a particular genetic disease before the actual onset of the disease.

e) PCR can be used for prenatal diagnosis of genetic diseases, e.g., sickle cell anaemia.

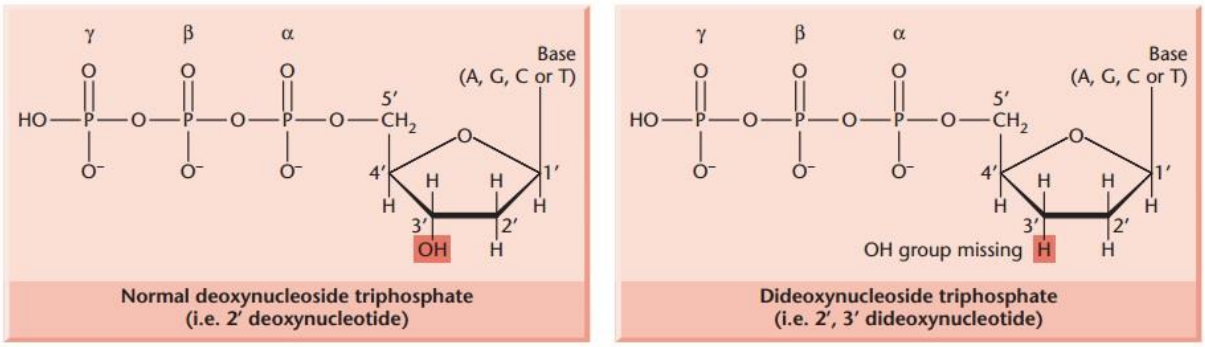
5.1.7. DNA Sequencing

The process of determining the nucleotide sequence/base sequence of a DNA molecule is called as DNA sequencing. Two methods have been developed to sequence DNA:

(1) The dideoxynucleotide chain termination method invented by Fred Sanger and Andrew Coulson.
(2) Automated DNA Sequencing

5.1.7.1. Sanger's dideoxy chain termination method

This method makes use of the mechanism of DNA synthesis by DNA polymerase. (a) DNA polymerase requires both a primer (a short oligonucleotide strand), to which nucleotides are added, and a template strand to guide selection of each new nucleotide. In cells, the 3'-hydroxyl group of the primer reacts with an incoming deoxynucleoside triphosphate (dNTP) to form a new phosphodiester bond. (b) The Sanger sequencing procedure uses dideoxynucleoside triphosphate (ddNTP) analogs to interrupt DNA synthesis. (The Sanger's method is also known as the dideoxy method.) When a ddNTP is inserted in place of a dNTP, strand elongation is halted after the analog is added, because it lacks the 3'-hydroxyl group needed for the next step. (c) The DNA to be sequenced is used as the template strand, and a short primer, radioactively or fluorescently labeled, is annealed to it. By addition of small amounts of a single ddNTP, for example ddCTP, to an otherwise normal reaction system, the synthesized strands will be prematurely terminated at some locations where dC normally occurs. Given the excess of dCTP over ddCTP, the chance that the analog will be incorporated whenever a dC is to be added is small. However, ddCTP is present in sufficient amounts to ensure that each new strand has a high probability of acquiring at least one ddC at some point during synthesis. The result is a solution containing a mixture of labeled fragments, each ending with a C residue. Each C residue in the sequence generates a set of fragments of a particular length, such that the different-sized



fragments, separated by electrophoresis, reveal the location of C residues.

This procedure is repeated separately for each of the four ddNTPs, and the sequence can be read directly from an autoradiogram of the gel. Because shorter DNA fragments migrate faster, the fragments near the bottom of the gel represent the nucleotide positions closest to the primer (the 5' end), and the sequence is read (in the 5' to 3' direction) from bottom to top. Note that the sequence obtained is that of the strand complementary to the strand being sequenced (template strand).

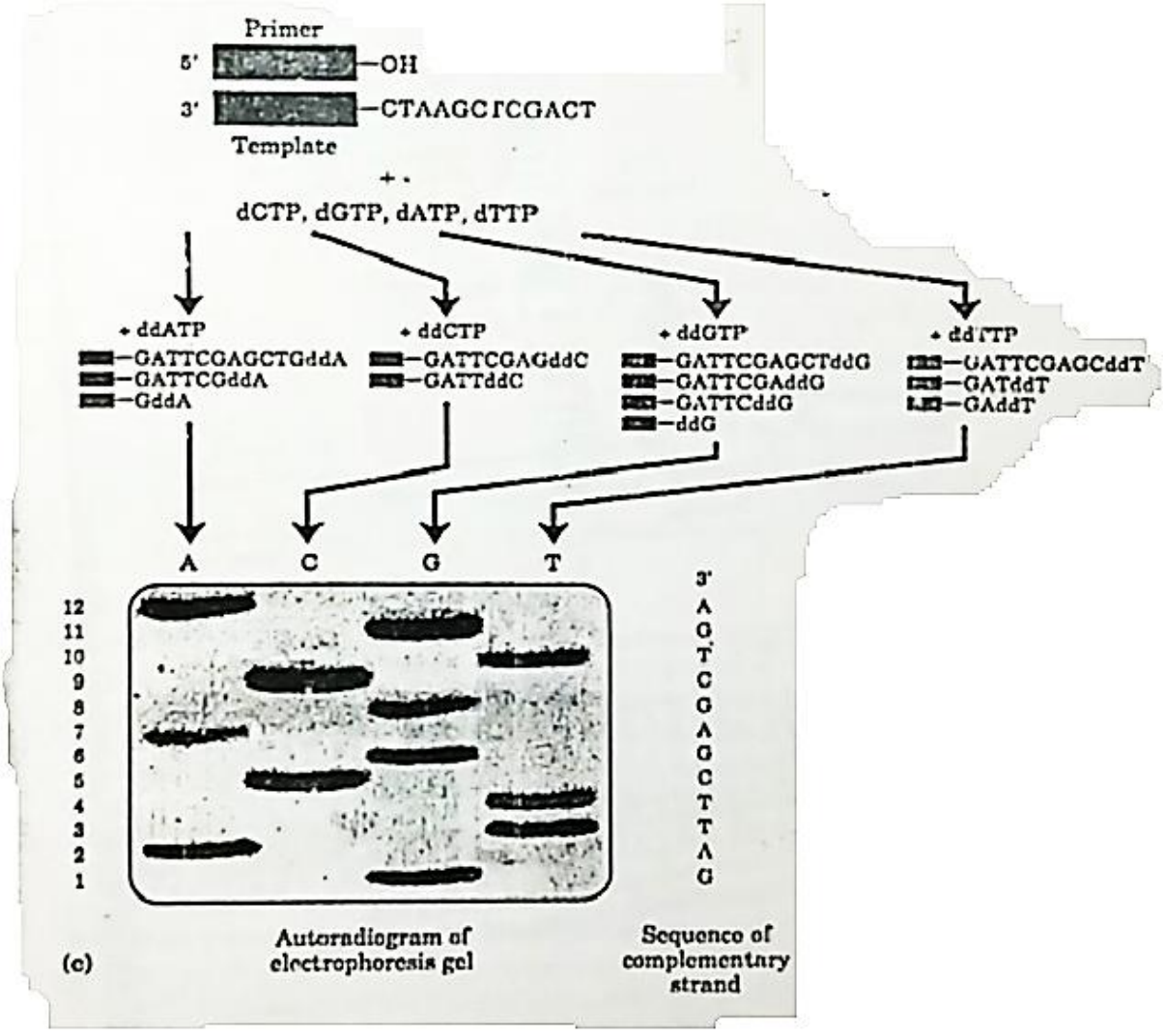
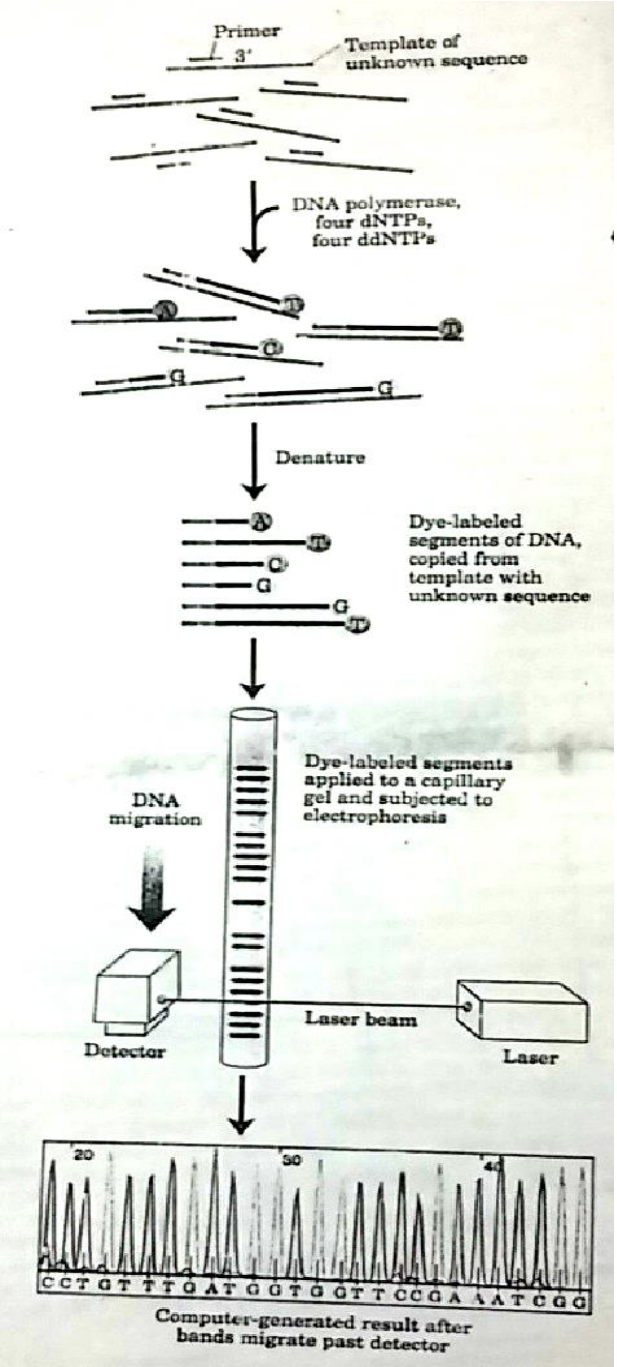


Fig. 14. Steps of Chain Termination Method

5.1.7.2. Automated DNA Sequencing/Single Tube Sequencing Method

DNA sequencing is readily automated by a variation of Sanger's sequencing method in which the dideoxynucleotides used for each reaction are labelled with a differently coloured fluorescent tag. This technology allows DNA sequences containing thousands of nucleotides to be determined in a few hours.



Each dideoxynucleotide used in the Sanger's method can be linked to a fluorescent molecule that gives all the fragments terminating in that nucleotide a particular colour. All four labelled ddNTPs are added to a single tube. The resulting coloured DNA fragments are then separated by size in a single electrophoretic gel contained in a capillary tube (a refinement of gel electrophoresis that allows for faster separations). All fragments of a given length migrate through the capillary gel in a single peak, and the colour associated with each peak is detected using a laser beam. The DNA sequence is read by determining the sequence of colours in the peaks as they pass the detector. This information is fed directly to a computer, which determines the sequence.

Fig. 15. Automated DNA Sequencing

CHAPTER-5.1

RECOMBINANT DNA TECHNOLOGY

5.1.1. MULTIPLE CHOICE QUESTIONS

1. Which Enzyme is used by bacteria for defense against viruses?
   a) DNA Polymerase
   b) DNA Ligase
   C) Restriction Endonuclease
   d) Primase

2. Which of these is called as the molecular vehicle?
   a) Host
   b) Restriction Enzyme
   c) Vector
   d) Gene of interest

3. Which vector is used for genome sequencing?
   a) M13
   b) YAC
   c) BAC
   d) Both B & C

4. By which method the recombinant vector enters into the host cell?
   a) Transition
   b) Transformation
   c) Conjugation
   d) None of the above

5. Which of these produces ssDNA?
   a) *p*BR322
   b) M13

c) BAC
d) YE*p*

6. Which enzyme is produced by lacZ gene?
   a) Beta Galactosidase
   b) Beta lactase
   c) Beta permease
   d) Acetylase

7. Which of these is not true for lambda phage vector:-
   a) It is linear and double stranded
   b) It has cos sites
   c) It follows lytic pathway
   d) It's insert size is 45 kbp

8. Which of these is not a selectable marker gene?
   a) LEU2
   b) GFP
   c) amp^R
   d) MCS

9. Which of these gene transfer methods may need calcium phosphate?
   a) Transformation
   b) Microinjection
   c) Biolistics
   d) Transfection

10. Which of these is a shuttle vector?
   a) YE*p*
   b) *p*BR322
   c) *p*UC19
   d) BAC

11. If your foreign DNA is of size 35 kbp, which vector would you use?
   a) BAC
   b) YAC
   c) Cosmid

d) lambda

12. A vector based on which virus was used in the first cloning experiment involving mammals?
    a) Adenovirus
    b) Papilloma virus
    c) SV40
    d) Retrovirus

13. Which microscope is required for microinjection?
    a) Electron Microscope
    b) Phage Contrast Microscope
    c) Fluorescent Microscope
    d) All can be used

14. How does a scientist select for a sensitive or negative trait following a transformation experiment?
    a) By Replica Plating
    b) By Blue White Screening
    c) Both can be used

15. Who found the transformation of *E.coli* cells upon treatment with chilled $CaCl_2$ solution?
    a) Mandel & Higa
    b) Herbert Boyer & Paul Berg
    c) Watson & Crick
    d) Stanley & Cohen

16. If a PCR reaction initiated with three molecules of a dsDNA template, after 35 cycles the amount of DNA will be:
    a) 3 x 35/2
    b) 3 x (2 x 35)
    c) 3 x (2/35)
    d) 3×2^{35}

17. From the options given below which vector is used between two hosts?

a) *p*BR322
b) *p*UC19
c) YE*p*
d) All of these

18. For performing Site directed mutagenesis, which vector will be required?
a) phage
b) cosmid
c) M13
d) BAC

19. What is the expansion of ddNTP?
a) Dedioxynucleotide triphosphate
b) Dideoxynucleoside triphosphate
c) Dideoxynucleotide triphosphate
d) Deoxynucleotide triphosphate

20. Which of these is not present in a YAC vector?
a) Centromere
b) Telomeric Sequence
c) cos site
d) Autonomously Replicating Sequence (ARS)

21. Which of these is not an animal vector?
a) SV40
b) Gemini Virus
c) Adenovirus
d) Papilloma Virus

22. Which of these enzymes can be used to prevent self-ligation?
a) *Eco*RI
b) DNA Ligase
c) *Hin*dIII
d) Alkaline Phosphatase

23. Which of these is used by bacteria to protect their own DNA from the action of restriction endonucleases?

a) Alkylation
b) Phosphorylation
c) Methylation
d) Hydrolysis

24. If you are using YE*p* vector having LEU2 selectable marker gene, what would be your media?
a) with/containing leucine
b) without leucine
c) None of these
d) can't say

25. Which of these vectors would be needed for template preparation for sanger's method of DNA sequencing?
a) Lambda phage
b) *p*BR322
c) M13
d) BAC

26. Which of these methods is used exclusively for gene transfer
in plants?
a) Electroporation
b) Microinjection
c) Biolistics
d) Transfection

27. What is the sequence of steps in a cycle of PCR?
a) Denaturation, Extension, Annealing
b) Extension, Annealing, Denaturation
c) Annealing, Denaturation, Extension
d) Denaturation, Annealing, Extension

28. Polymerase Chain Reaction was invented by:
a) Edward Southern
b) Kary Mullis
c) Fredrick Sanger
d) Paul Berg

29. If you are interested in amplifying a specific region of genome of an organism, which technique would you use?
    a) Sanger's dideoxy chain termination
    b) Polymerase Chain reaction
    c) Replica Plating
    d) Blue White screening

30. If you are interested in amplifying a specific region of genome of an organism, which of the following would actually help in such targeted amplification?
    a) Probes
    b) Primers
    c) dNTPs
    d) ddNTPs

31. Which vector is used for blue white screening?
    a) BAC
    b) YAC
    c) *p*BR322
    d) *p*UC19

32. What was labeled with fluorescent tag in the single tube DNA sequencing method?
    a) Primer
    b) dNTP mix
    c) ddNTP
    d) None of these

33. In DNA sequencing method, chain elongation terminates due to lack of ____ in ddNTP.
    a) 2' hydroxyl group
    b) 3' hydroxyl group
    c) 5' Phosphate group
    d) All of these

34. In Sanger's chain termination method, lack of formation of which bond terminates the chain elongation?

a) Phosphoester Bond
b) Phosphodiester Bond
c) Glycosidic Bond
d) Peptide Bond

35. Expand RFLP.
a) Restriction fragment length polymorphism
b) Restricted fragment linear polymorphism
c) Regenerated fragment length polymorphism
d) Regenerated fragment linear polymorphism

36. Which of these is a bacterial vector compatible with plant cells?
a) Gemini Virus
b) Cauliflower Mosaic Virus
c) Ti plasmid
d) Tobacco mosaic virus

37. If you are treating bacterial host cells with calcium chloride for facilitating introduction of rDNA, which of these methods you are using?
a) Transformation
b) Electroporation
c) Microinjection
d) Biolistic

38. If you are coating your DNA of interest onto the surface of microscopic gold particles for introduction in host cell, which of these methods you are using?
a) Transformation
b) Transfection
c) Microinjection
d) Biolistic

39. Suppose you want to insert your gene of insert in *p*BR322 vector within the tetracycline resistance selectable marker gene, by which of the following restriction enzymes you would isolate the gene of interest?
a) *Alu*I

b) *Pst*I
c) *Eco*RI
d) *Bam*HI

40. If you are doing replica plating using *p*BR322 vector for selection of transformed recombinant cells, in which plate would the transformed recombinant cells grow?
a) master plate
b) replica plate
c) Both the above plates
d) None of the above plates.

41. Which of the following types of restriction enzymes is used in recombinant DNA technology?
a) Type I
b) Type II
c) Type III
d) All of these

42. Which of these is the correct sequence of steps of gene cloning?
a) Transformation, Restriction Enzyme digestion, Ligation, Selection.
b) Restriction Enzyme digestion, Selection, Ligation, Transformation
c) Restriction Enzyme digestion, Ligation, Selection, Transformation
d) Restriction Enzyme digestion, Ligation, Transformation, Selection

43. What is the advantage of MCS or polylinker site in vector?
a) increases flexibility of choice of restriction enzymes
b) decreases flexibility of choice of restriction enzymes

c) increases flexibility of choice of selectable marker genes

d) decreases flexibility of choice of selectable marker genes

44. Which of these sequences can be cut by a type II restriction enzyme?

a) ATGCGTCGTGTCGTG
b) GTGCGATGACGTGCG
c) ATGGAATTCGTGCGTA
d) GTGATGACGTAGCGTG

45. Which of the following can be used to create a recombinant DNA?

a) A fruitfly gene isolated by *Hin*dIII digestion and a human gene isolated from *Eco*RI digestion.
b) A fruitfly gene isolated by *Hin*dIII digestion and a human gene isolated from *Hin*dIII digestion.
c) A fruitfly gene isolated by *Hin*dIII digestion and a human gene isolated from *Eco*RI digestion.
d) None of these

46. The first recombinant DNA was developed by whom?

a) Boyer, Berg, Chang, Cohen.
b) Boyer, Mullis, Sanger
c) Arber, Smith and Nathans
d) Linus Pauling, Winkler

47. The first type II restriction enzyme was isolated by whom?

a) Boyer, Berg, Chang, Cohen.
b) Boyer, Mullis, Sanger
c) Arber, Smith and Nathans
d) Linus Pauling, Winkler

48. What is the source of DNA ligase for recombinant DNA technology?

a) T4 bacteriophage
b) Calf intestine
c) Bacteria
d) Plants

49. The natural genetic engineer of plants is:-

a) *Alcaligenes eutrophus*
b) *Agrobacterium tumefaciens*
c) *Bacillus thuringiensis*
d) *Bacillus amyloliquefaciens*

50. What is the role of GFP in recombinant DNA technology?

a) acts as Selectable marker gene
b) performs restriction digestion
c) increases rate of transformation in host cells
d) all of these

ANSWERS OF MCQs

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

5.1.2. ASSERTION REASON QUESTIONS

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

1) Assertion: Type III restriction enzymes are used in recombinant DNA technology.
Reason: Type III enzymes recognise and cut the DNA within a recognition sequence.

2) Assertion: Vectors are treated with alkaline phosphatase.
Reason: This enzyme avoids self ligation of vectors by removing their 5' phosphate group.

3) Assertion: YEP is used for both prokaryotes and eukaryotes cells.
Reason: It is a shuttle vector.

4) Assertion: PCR based diagnosis is faster, safer and more specific.
Reason: As it does not use live pathogens.

5) Assertion: PCR uses Taq DNA polymerase enzyme.
Reason: It is isolated from a thermostable bacteria.

6) Assertion: *p*BR322 based true recombinants will be tetracycline sensitive if the vector was disarmed with Bam H1 restriction endonuclease enzyme.

Reason: Insert fails to show insertional inactivation in tetracycline resistance gene in recombinant cells.

7) Assertion: M13 is used in DNA sequencing and site directed mutagenesis techniques.
Reason: It produces replicative forms.

8) Assertion: The ends of lambda phage DNA are sticky and called *cos* sites.
Reason: 12 bases on each end are unpaired and complementary to each other.

9) Assertion: *p*UC19 is an expression vector.
Reason: *p*UC19 has signals for transcription and translation in given host.

10) Assertion: *Agrobacterium tumifaciens* is known as natural genetic engineer.
Reason: It inserts a part of its own DNA sequence into host plant cell.

ASSERTION- REASON QUESTIONS

ANSWER KEY

Q.NO.	**1**	**2**	**3**	**4**	**5**
ANSWER	d	a	a	a	a
Q.NO.	**6**	**7**	**8**	**9**	**10**
ANSWER	c	b	a	a	a

5.1.3. VERY SHORT ANSWER QUESTIONS

1) Name any two scientists involved in designing the first recombinant DNA molecule.

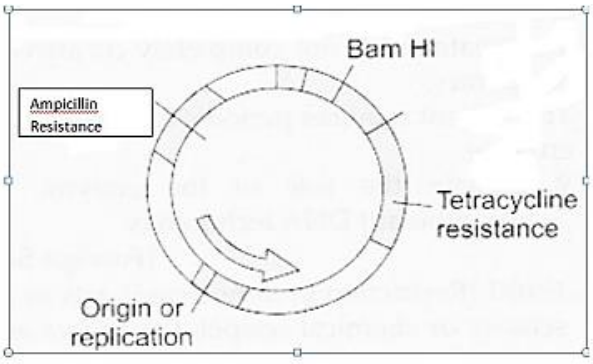
Ans. Paul Berg, Herbert Boyer, Annie Chang and Stanley Cohen.

(Any two)

2) Answer the following:-

a) Which vector was used in the first cloning experiment involving mammalian cell?

b) Identify the vector shown below.



Ans. a) Simian Virus 40

a) *p*BR322

3) Answer the following:

a) Explain R-M system existing in bacteria.

b) How does a modification enzyme protect bacterial DNA from its own restriction enzymes?

Ans. a) Restriction- Modification system: The main components include restriction enzyme (R), which cuts specific non-methylated DNA sequences thereby restricting the propagation of any external DNA like that of bacteriophage inside bacterial cells, and the methyl transferase or methylase (M), which protects bacterial own DNA sequences from the action of its own restriction enzyme(s).

b) By methylation of host DNA so that it become resistant to the action of restriction enzymes.

4) How can LEU2 gene be used as a selectable marker?

Ans. LEU2 gene codes for an enzyme required for the biosynthesis of amino acid leucine in yeast cells. Yeast cells having this plasmid can grow on a minimal medium lacking leucine and hence can be selected out of other yeast cells which do not contain this gene.

5) Specify one advantage of developing vectors based on M13 vector.

Ans. Multiple single stranded copies of the initial double stranded gene can be obtained while gene cloning via M13 vector.

5.1.4. SHORT ANSWER QUESTIONS

1) Listed below are four different single strands of DNA. Which of these would you expect to be cleaved by a restriction endonuclease? Give reason.

(a) ACTCCAGAATTCACTCCG

(b) ACTCCACTCCCGACTCCG

(c) GCCTCAAAGCTTGCCTGA

(d) GAGCGGTTTATCTGAGCAG

Ans. (a) ACTCCAGAATTCACTCCG

(c) GCCTCAAAGCTTGCCTGA

These sequences have recognition palindromic sequences for restriction enzymes a) *Eco*RI and c) *Hin*dIII

2) Explain various steps involved in a recombinant DNA technology experiment.

Ans. Steps involved: 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 and allowing them to multiply thereby multiplying the rDNA molecules.

3) Students of Class XII visited Microbial Type Culture Collection, Chandigarh and observed microbial cultures of *Providencia stuartii*, *Streptomyces albus* and *Haemophilus aegyptus*. Name the restriction enzymes obtained from them and also specify their restriction sites.

Ans. a) Source- *Haemophilus aegyptus*
RE- *Hae*III
Res. Site- 5' G-G-C-C 3'

b) Source- *Providencia stuartii*
RE- *Pst*I
Res. Site- 5' C-T-G-C-A-G 3'

c) Source- *Streptomyces albus*
RE- *Sal*I
Res. Site- 5' G-T-C-G-A-C 3'

4) Selection is an important step in genetic engineering. You are given ampicillin and tetracycline antibiotics. Using these antibiotics, which selection technique could be used to differentiate between recombinant and non-recombinant cells?

Ans. Transformed host cells are first plated on an agar medium containing that antibiotic the resistance gene for which is intact, and the DNA fragment is not inserted, within this gene. Therefore, host cells having the recombinant DNA are expected to be resistant to this antibiotic. This step eliminates non-transformed cells; the resulting colonies will possess

either recombinant or unaltered vector. The colonies so obtained are then replica-plated on agar plates containing the other antibiotic (within the gene for which the DNA insert is placed); all the colonies that develop on this plate will contain the unaltered vector. Therefore, the antibiotic sensitive colonies are identified and recovered from the master plate; these colonies will have recombinant DNA. This entire process may take 1-2 days.

5.1.5. LONG ANSWER QUESTIONS

1) Few restriction enzymes break the phosphodiester bond in such a manner that single stranded overhanging ends are generated in the DNA strand. *Eco*RI is one such restriction enzyme.

(a) Write the sequence for restriction site for enzyme *Eco*RI. Give a name to the type of ends generated here. Are all the restriction sequences palindromic in nature?

(b) Explain about any two vector-less methods that allow DNA to enter host cells.

(c) Why is small size desirable in a cloning vehicle?

Ans. (a) Restriction site of EcoRI is 5'-GAATTC-3'
The ends generated will be called sticky/cohesive ends.
No, all the Restriction sequences may not be palindromic.

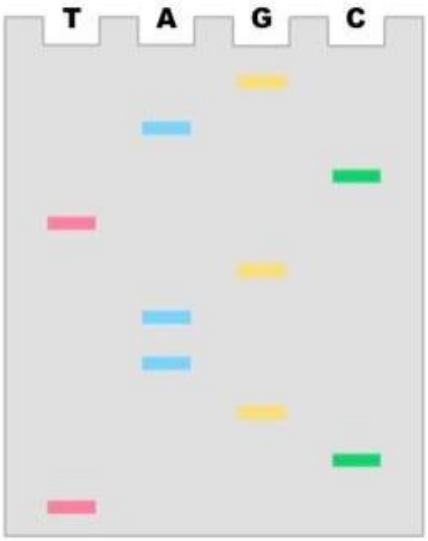
(b) Microinjection method can be used to inject foreign DNA into plant and animal cells' nucleus.

Biolistic method makes use of particle gun to bombard gold/tungsten bullets coated with DNA so as to deliver DNA into host cells.

(c) Small size of vector facilitates entry of recombinant molecules into the host cells. Moreover, small vectors

are more stable and less likely to break during various manipulations taking place in gene cloning process.

2) Given below is the autoradiogram obtained as a result of Sanger's method of DNA sequencing. Based on this answer the following questions.



(a) Read and write the original/template DNA sequence from the autoradiogram below.

(b) Define the principle and steps of this technique.

Ans. (a) 3' AGCTTCAGTC 3'

(b)Principle – When a ddNTP gets incorporated in the growing DNA chain during DNA replication, the extension stops as due to non-availability of 3'

hydroxyl group on the ddNTP present at the growing

end, incoming nucleotide cannot join.

Steps-

This method makes use of the mechanism of DNA synthesis by DNA polymerase. (a) DNA polymerase requires both a primer (a short oligonucleotide strand), to which nucleotides are added, and a template strand to guide selection of each new nucleotide. In cells, the 3'-hydroxyl group of the primer reacts with an incoming deoxynucleoside triphosphate (dNTP) to form a new phosphodiester bond. (b) The Sanger sequencing procedure uses dideoxynucleoside triphosphate (ddNTP) analogs to interrupt DNA synthesis. (The Sanger's method is also known as the dideoxy method.) When a ddNTP is inserted in place of a dNTP, strand elongation is halted after the analog is added, because it lacks the 3'-hydroxyl group needed for

the next step. (c) The DNA to be sequenced is used as the template strand, and a short primer, radioactively or fluorescently labeled, is annealed to it. By addition of small amounts of a single ddNTP, for example ddCTP, to an otherwise normal reaction system, the synthesized strands will be prematurely terminated at some locations where dC normally occurs. Given the excess of dCTP over ddCTP, the chance that the analog will be incorporated whenever a dC is to be added is small. However, ddCTP is present in sufficient amounts to ensure that each new strand has a high probability of acquiring at least one ddC at some point during synthesis. The result is a solution containing a mixture of labeled fragments, each ending with a C residue. Each C residue in the sequence generates a set of fragments of a particular length, such that the different-sized fragments, separated by electrophoresis, reveal the location of C residues.

This procedure is repeated separately for each of the four ddNTPs, and the sequence can be read directly from an autoradiogram of the gel. Because shorter DNA fragments migrate faster, the fragments near the bottom of the gel represent the nucleotide positions closest to the primer (the 5' end), and the sequence is read (in the 5' to 3' direction) from bottom to top. Note that the sequence obtained is that of the strand complementary to the strand being sequenced (template strand).

3)Expand PCR. What are the three basic steps of a typical PCR reaction? Explain in detail along with a suitable diagram.

Ans. PCR- Polymerase Chain Reaction

Process:-

The process of PCR consists of a series of reaction/amplification cycles and it is carried out *in vitro*. It utilizes the following : **(1)** a DNA preparation containing the desired DNA segment to be amplified (target sequence), **(2)** two oligonucleotide primers (about 20 bases long) specific, i.e., complementary, to the two 3'- borders (the sequences present at the 3'-ends of the two strands) of the desired segment, **(3)** the four deoxynucleoside triphosphates,

viz., dTTP (deoxythymidine triphosphate), dCTP (deoxycytidine triphosphate), dATP (deoxyadenosine triphosphate) and dGTP (deoxyguanosine triphosphate), and **(4)** a heat stable DNA polymerase, *e.g., Taq* polymerase (isolated from the bacterium *Thermus aquaticus*).

Steps of PCR:-

At the start of PCR, the DNA from which a segment is to be amplified, an excess of the two primer molecules, the four deoxynucleoside triphosphates and the DNA polymerase are mixed together to form a reaction mixture that has appropriate quantities of Mg^{2+}.

A single PCR amplification cycle involves the following three basic steps (Fig. 13):

a) Denaturation

The reaction mixture is first heated to a temperature between 90-98ºC (commonly 94ºC) that ensures DNA denaturation (separation of both of the strands of DNA). This is the denaturation step. The duration of this step in the first cycle of PCR is usually 2 minutes at 94ºC.

b) Annealing

The mixture is now cooled to a temperature (generally 40-60ºC) that permits annealing of the primer to the complementary sequences in the DNA; these sequences are located at the 3'-ends of the two strands of the desired segment. This step is called annealing. The duration of annealing step is usually 1 minute during the first as well as the subsequent amplification cycles of PCR. Since the primer concentration is kept very high relative to that of the template DNA, primer-template hybrid formation is greatly favoured over re-annealing of both the template strands.

c) Primer Extension/Polymerisation

The temperature is now so adjusted that the DNA polymerase synthesizes the complementary strands by utilizing 3'-OH of the primers; this reaction is the same as that occurs *in vivo* during

replication of the leading strand of a DNA duplex. The primers are extended towards each other so that the DNA segment lying between the two primers is copied; this is ensured by employing primers complementary to the 3'-ends of the segment to be amplified. The duration of primer extension is usually 2 minutes at 72ºC.

The completion of the extension step completes the first amplification cycle of PCR. Each cycle of PCR ordinarily takes 4-5 minutes. To begin the second cycle, the DNA is again heated to convert all the newly synthesized DNA into single strands, each of which can now serve as a template for synthesis of more new DNA. Thus the extension products of one cycle can serve as a template for subsequent cycles and each cycle doubles the amount of DNA from previous cycle. As a result, from a single template DNA molecule, it is possible to generate 2^n molecules after n number of cycles. Usually, 20-30 cycles are carried out in most PCR experiments. In case of automated PCR machines, called **thermal cyclers,** the researcher has to only specify the number

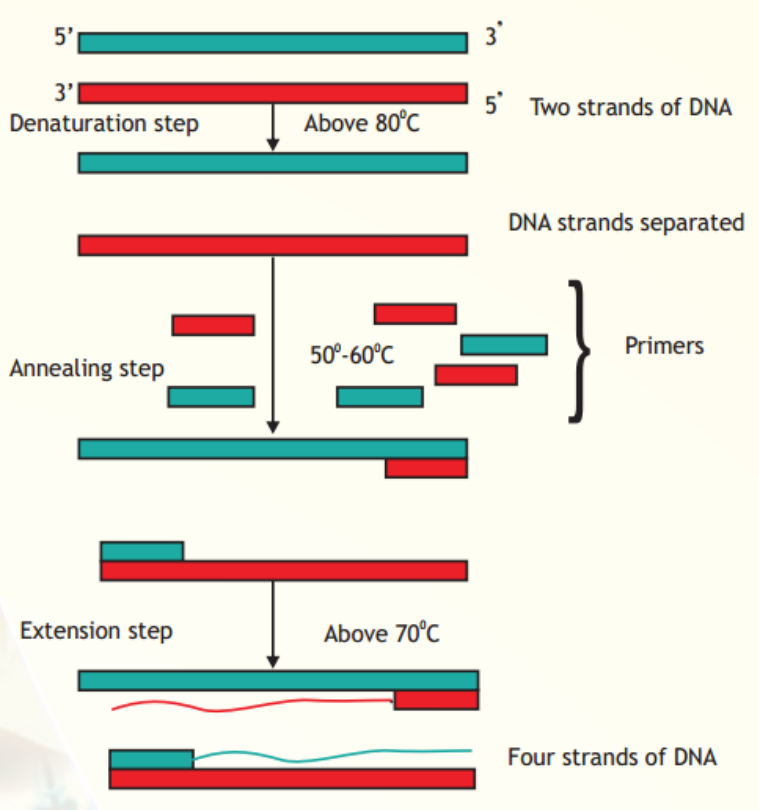


Fig. A schematic representation of the three steps performed during each reaction cycle of PCR.

and duration of cycles, etc. after placing the complete reaction mixture for incubation, and the machine performs the entire programme of operations precisely.

5.1.6 CASE BASED QUESTIONS

1) Forensics, short for forensic science, is the application of science in a legal setting. Biotechnology is used by forensic scientists to collect or process trace evidences such as hair, skin, blood or semen samples, which are found at crime scene(s). An important aspect of modern forensics is the use of DNA profiling, or genetic fingerprinting. Sources of DNA include blood, hair, semen, saliva, bone and tissue. Every person has a unique DNA profile. The only exception to this are monozygotic twins. The chemical structure of everyone's DNA is the same. There are so many millions of base pairs in each person's DNA that every person has a different sequence. Using these sequences, every person could be identified solely by the sequence of their base pairs. However, because there are so many millions of base pairs, the task would be very time-consuming. Instead, scientists are able to use a shorter method, because of repeating patterns in DNA. With the help of a technique that amplifies DNA, which is used to make millions of exact copies of DNA from a biological sample, it is possible to execute DNA fingerprinting even analysing a very small quantity of DNA.

(i) PCR is a:-

(a) DNA degradation technique
(b) DNA sequencing technique
(c) DNA amplification technique
(d) All of these

Ans. (c) DNA amplification technique

(ii) How many DNA duplexes are obtained from one DNA duplex after 5 cycles of PCR?

(a) 25
(b) 10
(c) 32

(d) 5

Ans. (c) 32

(iii). Primers used for the Polymerase chain reaction are:-

(a) Single stranded DNA oligonucleotide

(b) Double stranded DNA oligonucleotide

(c) Single stranded RNA oligonucleotide

(d) Double stranded RNA oligonucleotide

Ans. (a) Single stranded DNA oligonucleotide

(iv). What is the function of primer?

(e) To identify the particular region of DNA to be copied by PCR.

(f) To copy DNA.

(g) To create DNA nucleotides.

(h) To maintain the temperature of the PCR reaction

Ans. (a) To identify the particular region of DNA to be copied by PCR.

OR

Choose the right combination of components required to set up PCR reaction-

(a) Template DNA, two primers, dNTPs and DNA ligase

(b) Template DNA, two primers, NTPs and DNA ligase

(c) Template RNA, two primers, NTPs and DNA polymerase

(d) Template DNA, two primers, dNTPs and DNA polymerase

Ans. (d) Template DNA, two primers, dNTPs and DNA polymerase

2. Advantage of using *E. coli* as host cells is that under optimal conditions the cells divide every 20 minutes making it possible to clone large amounts of foreign DNA and if the appropriate signals are incorporated into the vector

large amounts of recombinant proteins are available for therapeutics and other uses.

For the expression of eukaryotic proteins, eukaryotic cells are often preferred because, to be functionally active, proteins require proper folding and post translational modifications such as glycosylation which is not possible in prokaryotic (*E. coli*) cells. Even cloned eukaryotic genes containing introns cannot be processed in E. coli thereby necessitating the use of only eukaryotic host cells. Yeast cells have been used extensively for functional expression of eukaryotic genes because of several features. Yeasts are the simplest eukaryotic organisms (unicellular) and like E. coli have been extensively characterized genetically, easy to grow and manipulate and large amounts of cloned genes or recombinant proteins can be obtained from yeast cultures grown in fermentors (large culture vessels). Plant and animal cells may also be used as hosts in rDNA experiments and cells can be grown in tissue culture or can be induced and manipulated to form whole organisms (creation of transgenic animals and plants).

i) Calculate the total number of *E. coli* cells formed from an initial population of 10 cells incubated in optimum conditions for 04 hours.

Ans: 10×2^{12} cells

ii) Mr. Raj attempted to clone and express erythropoietin gene in E. coli. The gene was cloned successfully but could not express itself. What could be the reason(s)?

Ans: a) Erythropoietin, being a eukaryotic gene, has both coding and non-coding region within it. After transcription, the primary transcript has to be modified post transcriptionally via intron splicing, capping and tailing. *E. coli*, being a prokaryote, is not able to perform such complex post transcriptional modifications.

b) Erythropoietin also needs enormous post translational modifications, i.e., glycosylation and protein folding, which is not possible in a prokaryotic system like *E. coli*.

iii) What control elements or regulatory sequences are required in an expression vector so as to drive expression of desired gene successfully in the host?

Ans: Promoter, Operator, Ribosome Binding Site

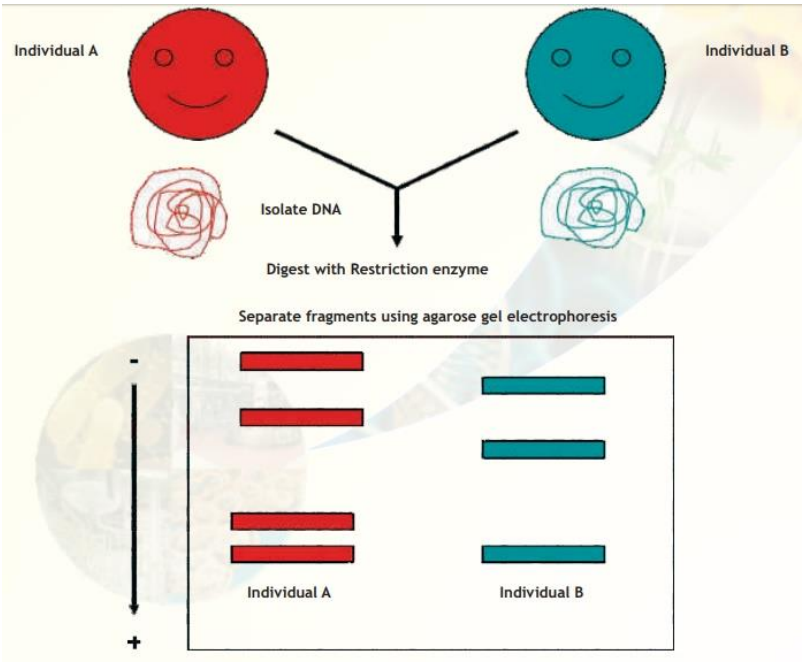
iv) Which device is used for large scale production of recombinant eukaryotic protein through microbial/host culture?

Ans: Bioreactor

v) Are genetically modified organisms (GMOs) and transgenic organisms are same? Justify.

Ans: No, the terms are often used interchangeably but the key difference between GMO and transgenic organism is that GMO is an organism that has an artificially altered genome, while the transgenic organism is a GMO that has an altered genome containing a DNA sequence or gene from a different species.

3. The variation in size (length) of the restriction enzyme generated fragments among individuals within a given species is termed RFLP. A schematic representation of how RFLPs are generated is given in the figure.



(i) The variation in size (length) of the restriction enzyme generated fragments among individuals within a given species is due to…….. ?

a) Usage of different restriction enzymes
b) Variation in charge between the DNA of two individuals
c) Size of their genome.
d) Difference in their DNA sequence and position of Restriction site

Ans: d)

(ii) Which among is not a property of Restriction site?

a) Palindromic
b) Generally 4-8 bp long
c) Restriction sites are universal
d) They always get cleaved via even cuts

Ans: d)

(iii) The individuals that have same RFLP are -

a) No identical wins
b) Brothers and sisters
c) Mother and daughter
d) Cloned animals

Ans: d)

(iv) RFLP technique is used in-

a) Parental diagnosis
b) Strain differentiation
c) Forensic science
d) All of these

Ans: d)

UNIT-5

PROTEIN AND GENE MANIPULATION

CHAPTER-5.2

PROTEIN STRUCTURE & ENGINEERING

5.2.1. Introduction to the World of Proteins

It is noteworthy that among the biomolecules you have studied, proteins have the maximum diversity in function.

The key to this enormous diversity is the unique structure of proteins.

Although all proteins are made up of 20 different amino acids the sizes and sequence combinations and variations of each protein leads to millions of unique 3-D structures and thereby functions.

Scientists have been striving to relate protein structure with function.

A number of human diseases are due to the deficiency or abnormal structure of proteins.

a) The lack of a particular subunit, alpha or beta of the oxygen carrying protein haemoglobin results in Thalassaemia, a devastating disease in which an infant cannot grow without repeated transfusions.
b) If the beta chain is present but with a substituent in one of the amino acid residues another debilitating condition called Sickle Cell Anaemia results which is endemic to certain parts of Africa.
c) The absence of an enzyme- Adenosine deaminase results in the birth of a severely immunocompromised baby who cannot last infancy (SCID).

d) More recently, it has been discovered that certain "rogue proteins" whose structure has been altered can result in diseases such as the Mad cow disease wherein the disease itself appears to be propagated by infectious proteins called "prions".

Different cells have specialised proteins for their unique functions in addition to the housekeeping proteins required for metabolism and generation of ATP.

5.2.2. Structure-Function Relationship in Proteins:-

5.2.2.1. Chymotrypsin, a proteolytic enzyme-

Chymotrypsin, which hydrolyses peptide bonds following bulky aromatic amino acid residues in polypeptides is actually synthesised in the pancreas and through the pancreatic duct released into the duodenum.

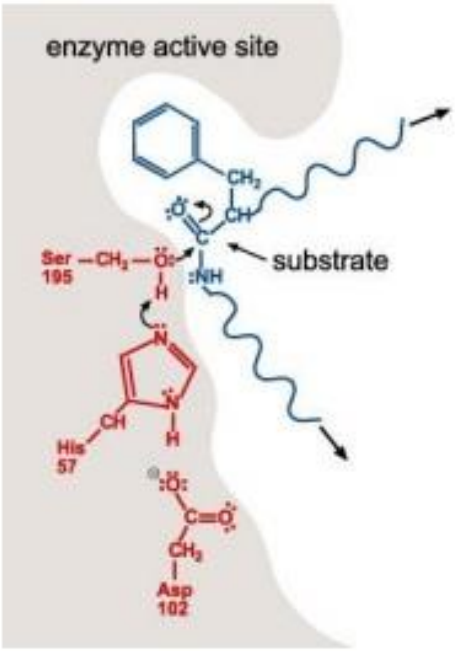
Chymotrypsin is synthesized as inactive harmless precursor, known as chymotrypsinogen (a type of zymogen), which is then activated, when required, only in the duodenum, its site of activity, via a process called in-situ activation. This activation in molecular terms results in an alteration in its shape so that it may now be able to interact with its substrate.

The enzyme chymotrypsin is made up of a linear chain of 245 amino acids interrupted into three peptides- A,B,C. The protein folds into a globular structure. In the 3-D structure of the enzyme, three important amino acid residues, his57, asp102 and ser195 come close together in space which allows a "charge relay system" to operate as indicated later. The negatively charged asp102 is able to hydrogen bond with the adjacent his57 partially borrowing the hydrogen ion from the latter. The his57 makes good its partial hydrogen ion loss to aspartate by attracting a

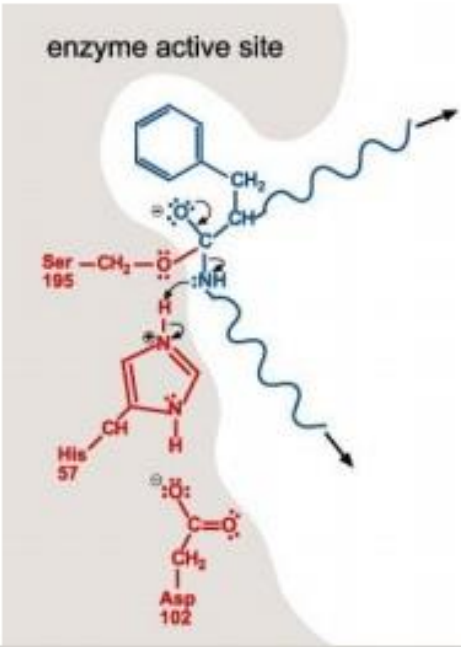
hydrogen ion from the adjacent ser195 through the his57 residue much like a relay race where the baton is passed from one member to another, the difference here being that the baton is a charge.

In chymotrypsin, ser195 becomes acidic due to the unique constellation of the three amino acid residues because the protein has folded uniquely in space. The specific

- polypeptide is in the active site
- an H^+ ion moves from the serine amino acid to histidine amino acid
- oxygen atom in serine forms a covalent bond to the carbon of one of the substrate's peptide bonds, it acts as a nucleophile

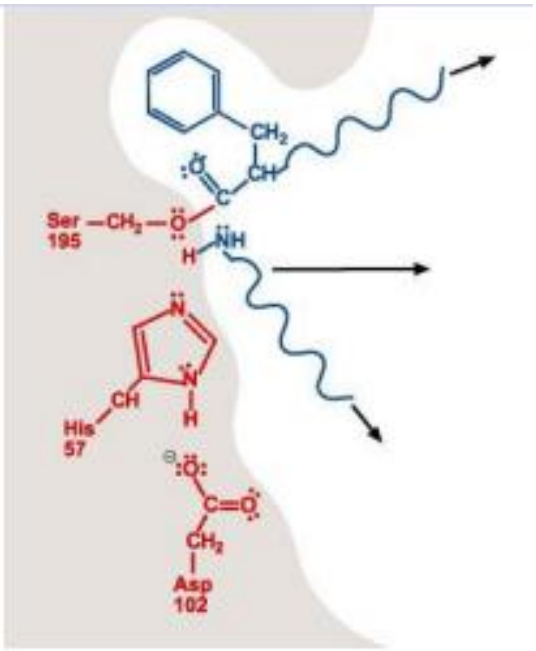


- Positive His-57 is stabilized by negative Asp-102
- Bond between the carbon and the nitrogen in the peptide bond is broken
- The nitrogen-containing group is stabilized by the formation of a bond to a hydrogen atom from His-57

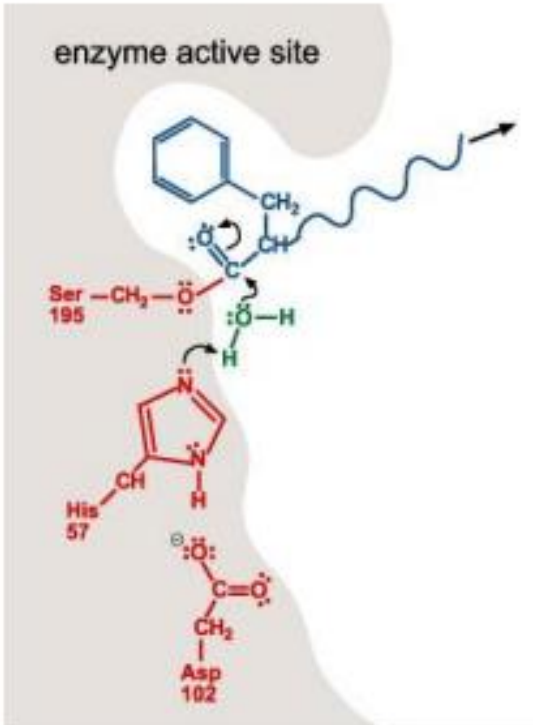


site of chymotrypsin (recall that the enzyme is specific to aromatic residues) is a large space created within the enzyme active site and lined by hydrophobic residues which therefore only allow bulky aromatic, hydrophobic amino acids to bind. This binding brings the susceptible peptide bond close to the attacking ser195 residue.

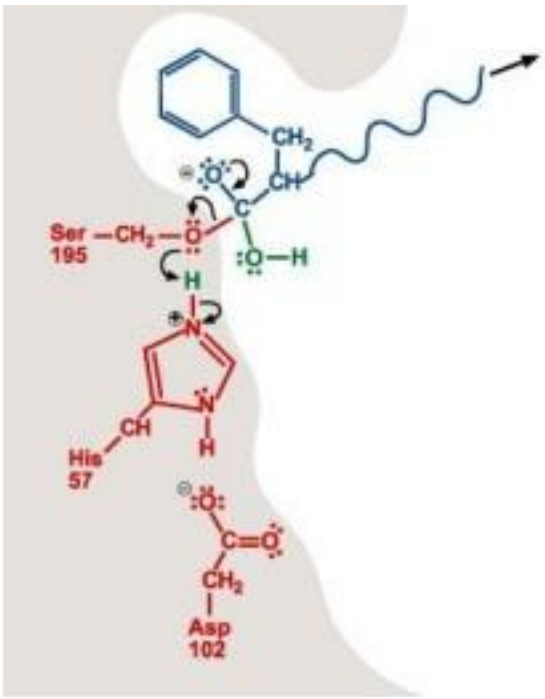
- The part of the polypeptide with nitrogen moves out of the active site (cleaved).



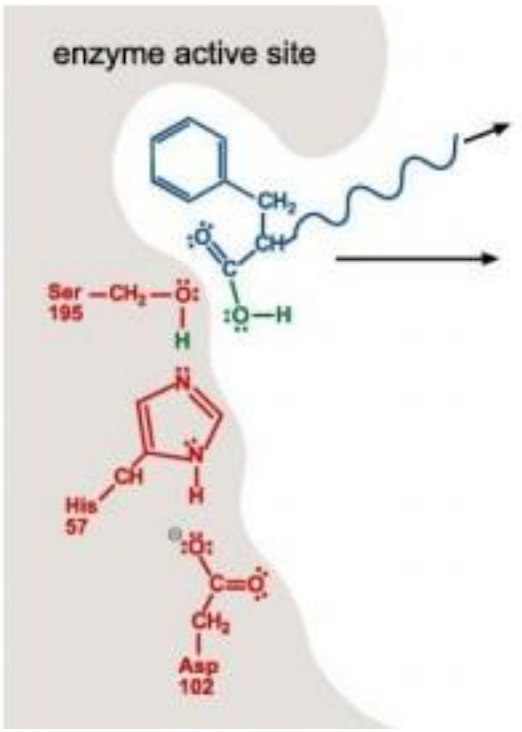
- Water molecule moves into the active site
- Oxygen in H_2O loses an H^+ ion to a nitrogen atom on His-57
- The oxygen atom then forms a bond with the carbon atom in the remaining portion of the substrate



- With double bond reformed, the bond between carbon and the oxygen of Ser-195 is broken
- The -OH group on Ser-195 is restored with a transfer of an H+ ion from His-57
- With this step, the Ser-195 and His-57 are both returned to their original forms



- The remaining portion of the substrate moves out of the active site
- Active site is back in its original form, ready to repeat the process.



This type of charge relay system mediated by reactive acidic serine residue also works in some other proteolytic enzymes like Trypsin, Subtilisin (a proteolytic enzyme found in *B. subtilis*, a bacterium), Thrombin (a proteolytic blood clotting factor) and the brain enzyme, Acetyl Choline Esterase.

5.2.2.2. Molecular Disease-Sickle cell anaemia

The red cells of the patient have a pronounced morphological change and resemble the shape of a farmer's sickle and thus the name of the disease.

Because these unusually shaped red cells have impaired oxygen carrying capacity and further get stuck in the small capillaries they lead to the anaemic conditions observed in patients.

One of the first attempts to study the molecular basis of sickle cell anaemia was to compare the electrophoretic mobility of normal (Hb) and sickle cell haemoglobin (scHb). On finding that Hb moved faster than scHb, Linus Pauling predicted that the latter differed in a charged amino acid. This was confirmed by V. M. Ingram in 1957 who pioneered a useful technique called protein finger printing in the famous Laboratory of Molecular Biology (LMB) at Cambridge, UK.

Protein Finger printing- Peptide Mapping

Each protein has a unique peptide map (2-D map) and hence serves as a fingerprint for the protein.

a) Pure Hb and scHb are taken separately into test tubes.
b) The Hb and scHb are digested with the proteolytic enzyme trypsin which cleaves the protein after basic amino acid residues Arg and Lys.
c) 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. Highly charged peptides will migrate more towards the anode/cathode.

d) The paper strips are dried, attached to larger squares of Whatman paper and chromatographed at right angles to the electrophoretic direction using a solvent system Butanol: Water:Acetic acid. In such a system peptides will separate based on their partition coefficient between the solvent and paper which is dependant on the relative hydrophobicity of the peptides. More hydrophobic peptides will move with the solvent to longer distances.

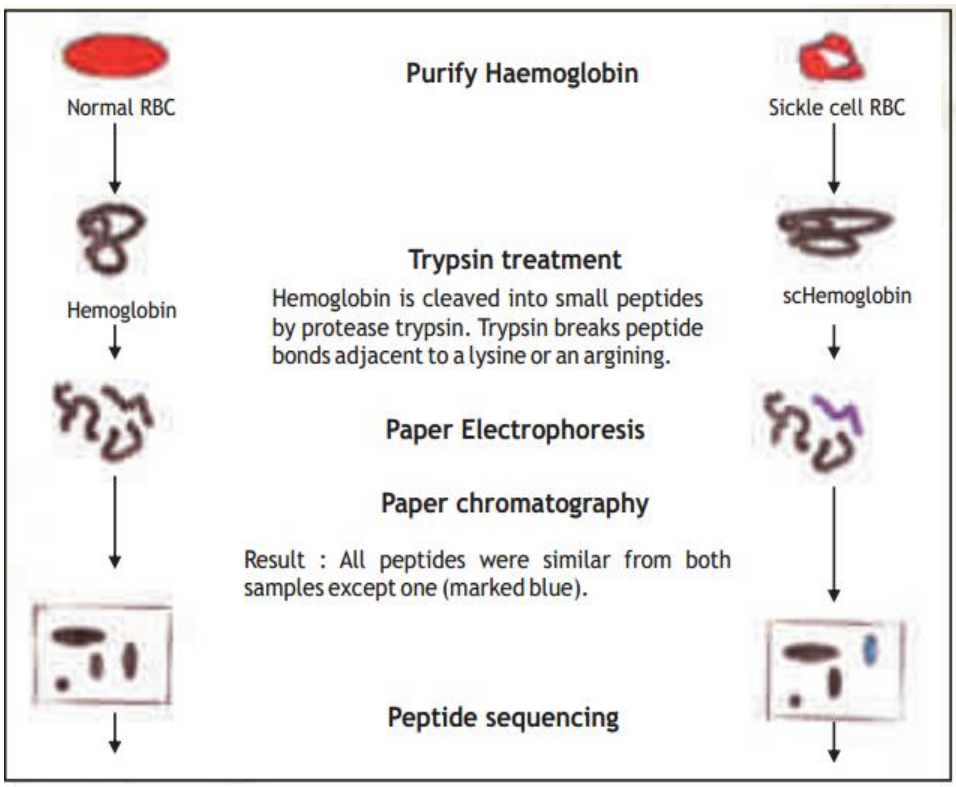


Fig. 1. Protein Finger Printing- Peptide Mapping

e) The chromatograms are dried and stained with a suitable visualisation reagent like Ninhydrin wherein peptide containing regions appear as orange yellow spots.

f) The peptide map for Hb and scHb are compared and it was found that one peptide was differently placed in the scHb map.

g) On examining this peptide and determining its amino acid sequence, Ingram found that it had a valine substitution for glutamic acid in the peptide.

The single substitution of valine for glutamic acid (at the 6th position of the beta chain) dramatically changes the structure of scHb making it form fibres within the RBC resulting in the deformation of the cell (sickling). Since the disease was due to a molecular alteration the term molecular disease was applied.

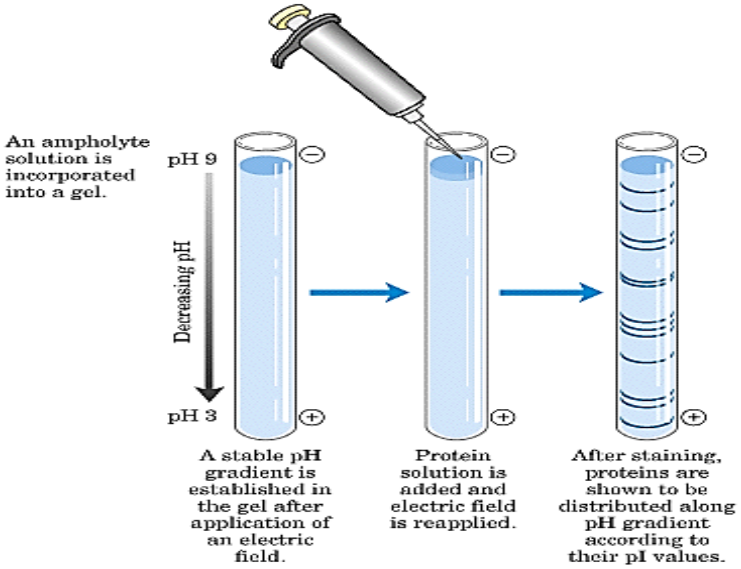
5.2.2.3. Two Dimensional Gel Electrophoresis

Two different techniques are combined in this procedure- Isoelectric focussing (IEF) and Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE).

In simple electrophoresis, the mobility of proteins is due to their charge, which is pH dependant. At its isoelectric pH (pI), a protein does not possess any charge and thus will not move in an applied electric field. This feature is exploited in the technique of IEF, which separates proteins on the basis of their different pI values.

Usually IEF is performed in thin tube gels. A pH gradient is set up within the IEF gel by the inclusion of polymeric buffers known as ampholytes.

A protein sample from a cell or any other source is then electrophoresed within these tubes wherein the different proteins separate and migrate to their pI zones. The tubes containing the separated proteins is then laid on a SDS-



PAGE slab gel and electrophoresis continued at right angles to the IEF direction.

In SDS-PAGE, proteins separate on the basis of their size and hence at the end of this electrophoretic run proteins are separated into 2-D patterns with high resolution as two properties of the proteins have been exploited in their separation- charge and size.

Proteins in the gels are stained with silver stains or other highly sensitive dyes and can be scanned and pictures stored into computer databases for analysis.

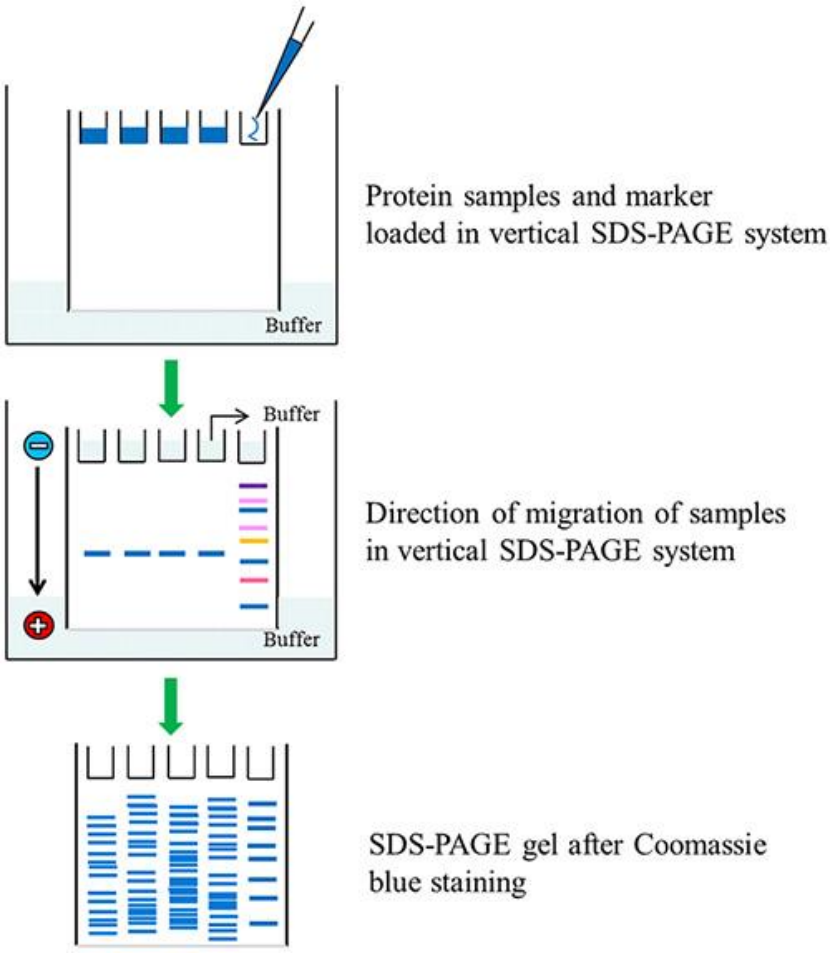
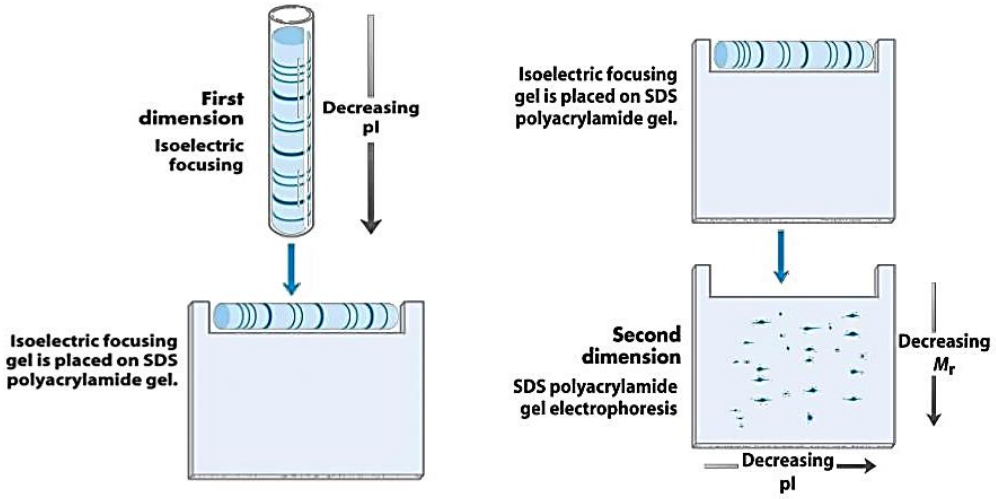


Fig: SDS PAGE Technique



5.2.3. Characterisation of Proteins:-

Techniques listed below characterise proteins with respect to properties such as mass, isoelectric charge, amino-acid sequence etc.

1) Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS/PAGE)
2) Protein Fingerprinting/Peptide Mapping
3) Two dimensional gel electrophoresis
4) Protein Sequencing
5) Mass Spectrometry

**The first three techniques have already been discussed in the above text.

4) Protein Sequencing

The process of determining the complete amino acid sequence of any given protein sample is known as protein sequencing.

There are two popular methods of the same:-

a) Sanger's Method
b) Edman's Degradation Method

a) .Sanger's Method-

In the middle of the last century, Dr. Frederick Sanger who developed the first sequencing reagent FDNB (fluoro dinitro benzene) and a general strategy for sequencing.

By using this method he was able to sequence the important hormone insulin which is required by diabetics and, more importantly, he demonstrated for the first time that proteins were linear polymers of amino acids.

For this work, he was awarded the Nobel Prize.

b). Edman's Degradation Method

Another protein chemist, Pehr Edman in 1950 developed another sequencing reagent, namely, Phenyl Iso thiocyanate (PITC), and procedure which is used in modern day sequenators as the procedure has been automated.

** Notably using the sequence of insulin established by Sanger a biotechnology company called Eli Lilli was able to develop recombinant human insulin which is the major source for insulin administration to diabetics worldwide.

5) Mass Spectrometry

Mass spectrometry is an analytical technique that determines the molecular weight of chemical compounds by separating molecular ions according to their mass/charge ratio (m/z).

It is extremely useful in obtaining protein structural information such as peptide mass or amino acid sequences.

The device used for mass spectrometry is known as Mass Spectrometer.

The molecular ions are generated either by a loss or gain of a charge (e.g. electron ejection, protonation or deprotonation).

After the ions are formed, they can be separated according to their m/z ratio and finally detected.

The process of ionisation, ion separation and detection in a mass spectrometer can provide molecular weight or even structural information.

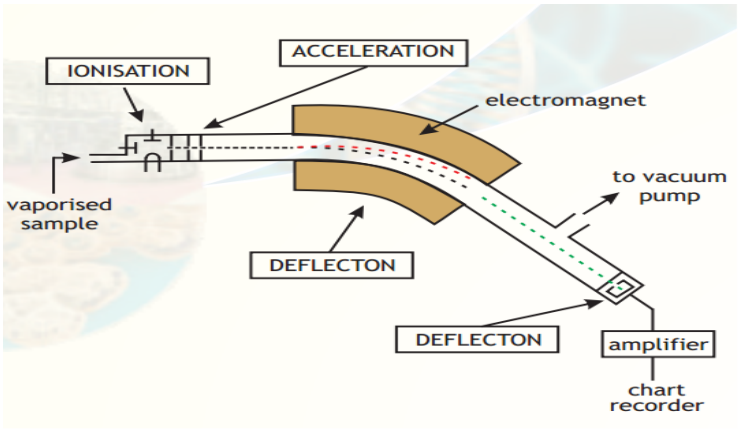


Fig-2 Parts of a Mass Spectrometer

Basically, a vapourised sample of a protein or peptide is introduced into the instrument wherein it undergoes ionisation. The charged molecules are then electrostatically propelled into a mass analyser (filter) which separates the ions according to their m/z ratio. The signal received upon detection of the ions at the detector is transferred to a computer which stores and processes the information and presents the output in the form of a mass spectrum, as indicated in fig-3, which can be analysed to deduce the molecular mass of the sample.

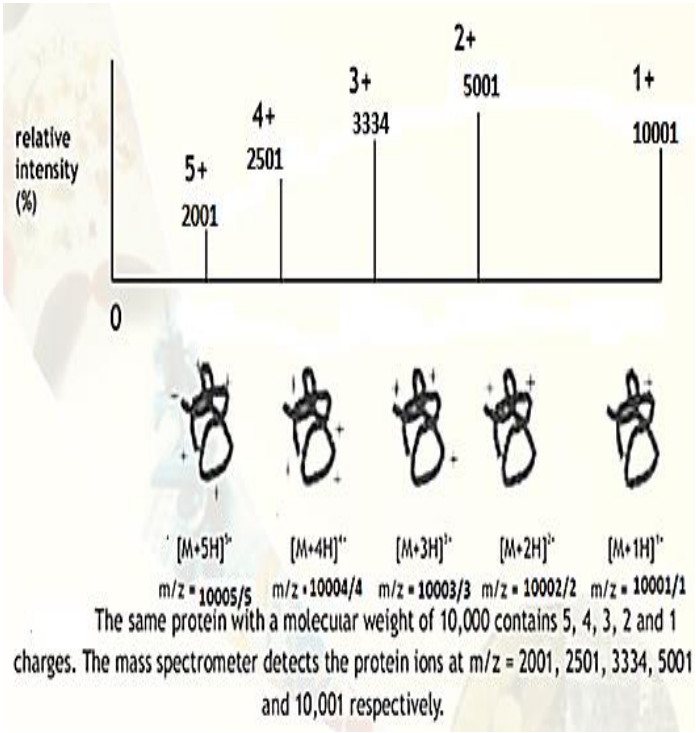


Fig-3 Mass Spectrum

The goal of mass spectrometric analysis of biomolecules like peptides and proteins is to create gas phase ions from polar charged molecules which are generally non-volatile. A popular method called Matrix Assisted Laser Desorption Ionisation (MALDI) is used to volatalise and protonate peptides and proteins.

In this procedure, the sample is transferred from a condensed phase to a gas phase with the help of a solid matrix. Ion formation in MALDI is achieved by directing a pulsed laser beam onto a sample suspended or dissolved in a matrix. The matrix plays a key role in this technique by absorbing the laser light energy and causing the matrix material to vaporise. In the gas phase, the matrix plays a role in sample ionisation. The charged molecules are directed by electrostatic lenses from the ionisation source to the mass analyzer .

5.2.4. Protein Based Products

a) Blood products and vaccines.
b) Therapeutic antibodies and enzymes.
c) Therapeutic hormones and growth factors.
d) Regulatory factors
e) Analytical application.
f) Industrial enzymes.
g) Functional non-catalytic proteins.
h) Nutraceutical proteins

a).Blood products and vaccines-

Factor VIII for treatment of Haemophilia A,
Factor IX for treatment of Haemophlia B,
Hepatitis B vaccine for prevention of hepatitis B.

b).Therapeutic antibodies and enzymes.

Monoclonal antibody preparation via Hybridoma Technology,

e.g. Monoclonal antibody OKT-3 used to prevent acute kidney graft rejection during kidney transplantation

Tissue plasminogen activator (t-PA) - a proteolytic enzyme used to digest blocks in arteries following myocardial infarction.

c).Therapeutic hormones and growth factors

Though insulin was prepared from the pancreas of cows and pigs, the ability to genetically transfer human insulin gene into bacteria and the ability to modify amino acid residues (protein engineering) has facilitated the development of modified forms which are faster acting like humulin. Humulin acts in 15 min unlike pig insulin which takes 3 hours.

Another growth factor- platelet derived growth factor has been approved for diabetics who develop skin ulcers.

d).Regulatory Factors

Various pharmaceutically important regulatory proteins collectively named as cytokines including interferons (alpha, beta & gamma), interleukins, tumor necrosis factor and colony stimulating factors are there.

Interferon alpha is used for treatment of Hepatitis C, beta for Multiple Sclerosis and gamma for Chronic Granulomatous disease.

e).Analytical Applications

Hexokinase for quantitative estimation of glucose in serum, Uricase for determining uric acid level in serum,
Horse radish peroxidise and alkaline phosphates in ELISA.

f).Industrial Enzymes

Alcalase is an enzyme used in the soap industry, papain is used in the beverage industry, glucose isomerase in the confectionary industry and chymosin is used in the cheese industry.

g).Functional non-catalytic proteins

Functional non-catalytic proteins are those which have properties such as emulsification, gelation, water binding, whipping and foaming as given in the table-

Functional Property	Mode of action	Food System
Whipping/Foaming	Forms stable film	Egg less cakes, desserts, whipped topping
Emulsification	Formation and stabilization of fat emulsions	Vegetarian sausages, salad dressings, coffee whiteners, soups, cakes, infant food formulas, biscuits.
Gelation	Protein matrix formation and setting	Meat, baked goods, cheeses
Viscosity	Thickening, water binding	Soups, gravies, salad dressings
Water binding	Hydrogen bonding of water; entrapment of water	Meats, sausages, cakes, breads
Solubility	Protein solvation	Beverages
Browning	Undergoes Maillard reaction (on heating, the amino groups of protein react with aldehyde groups of sugars)	Breads, biscuits, confections, sauces
Flavour/Aroma	Lactose reacts with milk proteins	Baked goods, biscuits, confectionaries, sauces, soups, dairy products.

Nutraceutical Proteins

(Nutrition + Pharmaceutical)

Nutraceutical proteins are those which are nutritious as well as have therapeutic function(s)/value.

Example- whey protein concentrates, lactose free milk (for lactose intolerant babies) and infant food formulations (Amul, Lactogen, etc.).

A typical composition of milk from buffalo, human and cow sources is given in the following table from which baby milk formulations can be made to suit an infant.

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

Table: Composition of milk from buffalo, human and cow

Besides use of milk as a nutritional source, it can also be used to set curd which is beneficial in the management of some types of intestinal infections and effectively improves digestion.

More recently, curd has also been used as a pro-biotic (administered with antibiotics) because it is a good source of beneficial bacteria which can colonise the intestinal tract.

Since time immemorial, whey (liquid part of curd) has been administered to the sick for the treatment of numerous ailments.

The spectrum of illnesses treated with whey include jaundice, infected skin lesions, genitor-urinary tract infections.

Using modern scientific research, it has been known that- Whey proteins result in the elevation of a tripeptide, namely, glutathione (gamma-glutamyl cysteinyl glycine) in cells. This peptide is a reducing compound and has a broad range of functions including detoxification of xenobiotics and protection of cellular

components from the effect of oxygen intermediates and free radicals. That's why, whey proteins have positive health effects.

5.2.5. Designing Proteins (Protein Engineering)

Considerable interest exists in the biotechnology industry for the engineering of proteins with increased stability when exposed to harsh conditions like elevated temperature, organic solvents and reactive chemicals, often encountered in the industrial processes.

The stability of a protein may be improved by substituting amino acids that either favour stabilising interactions in a folded protein or destabilising interactions in an inactive protein.

Numerous proteins and enzymes have been engineered in order to improve their properties like thermal and pH stability, solvent tolerance and solubility, catalytic potency, etc.

a) Improving laundry detergent Subtilisin

Subtilisin (27 kD) is a protease produced by bacteria that can digest a broad range of proteins that commonly soil clothing. The enzymatic activity of subtilisin is contributed by a catalytic triad, i.e., Ser221, His64 and Asp32 similar to chymotrypsin. Replacement of all three residues with alanine either singly or in combination results in significant loss of activity .

Subtilisin represents the largest industrial market for any enzyme. To improve the efficiency of laundry detergents, detergent manufacturers supplement subtilisin in their products with various catchy slogans on the detergent box such as "stain cutter" or "biologically active enzymes".

The native enzyme subtilisin is easily inactivated by bleach (up to 90%). Careful studies showed that this inactivation was due to oxidation of the amino acid residue Methionine222 in the protein molecule.

Using site-directed mutagenesis of the subtilisin gene in *E. coli*, this methionine was substituted by a variety of other amino acids and the enzyme activity measured in the presence of bleach. It was

observed that substitution of Met222 with Ala222 was the best in terms of activity and stability. Nowadays, many laundry detergents contain cloned, genetically engineered or recombinant subtilisin.

b) Creation of Novel Proteins

The specific sequences of amino acids in the protein which stimulate immune response are known as epitopes. A recombinant vaccine based on selected epitopes provides an optimal design, scope for micromanipulation, unhindered supply and safety needed for an effective vaccine. Working on these lines, a novel synthetic gene has been assembled as a first step towards developing a subunit vaccine against Hepatitis B virus.

c) Improving nutritional value of cereals and legumes

Deficiency in seeds of certain essential amino acids render the cereal grains or legumes unsuitable for a balanced diet. Supplementation of diet with essential amino acids from other sources therefore becomes essential.

The modern day approach for overcoming the nutritional deficiencies of seeds would be to engineer genes that would encode storage proteins with more of the nutritionally desirable amino acids either by inserting additional amino acids or substituting existing amino acids with new ones.

Whey protein is superior to other sources especially with regard to branched amino acids- ile, leu, val, lys and trp.

BCAAs (Branched Chain Amino Acids)-

The branched chain amino acids (BCAA) are essential for the biosynthesis of muscle proteins. They help in increasing the bio-availability of high complex carbohydrates intake and are absorbed by muscle cells for anabolic muscle building activity.

One of the theories is that during exercise the BCAAs are released from the skeletal muscle; the carbon skeleton part is used as fuel and the nitrogen part is used to make alanine which then goes to the liver where it is turned into glucose for

energy . So for athletes who want to protect their existing mass, the idea is to take BCAA enriched foods before and after excercise. BCAAs reduce muscle breakdown and act as an energy source before and after exercise. Hence while maintaining exercise performance and delaying exhaustion BCAAs are very important for muscle growth.

Biological value (BV) measures the amount of protein nitrogen that is retained by the body from a given amount of protein nitrogen that has been consumed. It has been observed that the BV of whey proteins is the highest compared to rice, wheat, soya and egg proteins.

Protein efficiency ratio (PER)- PER is used as a measure of growth expressed in terms of weight gain of an adult by consuming 1g of food protein. The PER value of the following proteins are arranged in decreasing order- whey, milk, casein, soya, rice, wheat.

5.2.1. MULTIPLE CHOICE QUESTIONS

1. The correct order for the basic features of a mass spectrometer is _____.

   a) Acceleration, deflection, detection, ionization
   b) Ionisation, acceleration, deflection, detection
   c) Acceleration, ionisation, deflection, detection
   d) Acceleration, deflection, ionisation, detection

2. Separation of ions in mass spectrometer take place on the basis of which of the following?
   a) Mass
   b) Charge
   c) Molecular weight
   d) Mass to charge ratio

3. The point mutation leading to sickle cell anaemia occurs:
   a) Sixth position in alpha chain of haemoglobin
   b) Sixth position in beta chain of haemoglobin

c) Sixth position in alpha chain of myoglobin
d) Sixth position in alpha chain of myoglobin

4. _____is a technique for separating different molecules by differences in their isoelectric point *p*I.

a. SDS-PAGE
b. 2D gel electrophoresis
c. Isoelectric focusing
d. Mass spectrometry

5. In isoelectric point-
a. net charge become high
b. net charge become zero
c. net charge become low
d. net charge has no relevance to isoelectric point

6. In case of 2-Dimensional Electrophoresis, proteins are treated with SDS which masks the proteins and provides them a negative charge. This indicates that:
a. The proteins are separated on the basis of mass-to-charge ratio in the second dimension
b. The proteins are solely separated on the basis of molecular weight in the second dimension
c. All the proteins now have approximately the same mass-to-charge ratio
d. The proteins are separated on the basis of charge to mass ratio in the second dimension

7. Proteins are solubilized and separated according to change *p*I in-
a. first dimension
b. second dimension
c. third dimension
d. at no dimension

8. SDS PAGE means-
a. sodium dodecyl sulfate polyamide gel electrophoresis
b. sodium didecyl sulfate polyamide gel electrophoresis
c. sodium dodecyl sulfate polyacrylamide gel electrophoresis

d. sodium didecyl sulfate polyacrylamide gel electrophoresis

9. Application of 2D gel electrophoresis is-
    a. analysis of cell differentiation
    b. detection of disease markers
    c. cancer research
    d. all the above

10. In SDS-PAGE migration of protein is affected by-
    a. charge of protein
    b. size of protein
    c. net charge of protein
    d. all the above

11. Proteins are separated in an SDS-PAGE experiment on the basis of their-
    a. positively charged side chains
    b. different isoelectric points
    c. molecular weight
    d. negatively charged side chains

12. The first attempts to study the molecular basis of sickle cell anaemia by comparing electrophobic mobility was done by-
    a. Linus Pauling
    b. V.M.Ingram
    c. O'Farrel
    d. Max Perutz

13. In an SDS-PAGE:-
    a. proteins are denatured by the SDS
    b. smaller proteins migrate more rapidly through the gel
    c. proteins have the same charge-to-mass ratio
    d. both a and b

14. For the treatment of Multiple sclerosis _____ is used.
    a.INFα b.INFβ c.INFπ d. INFμ

15. When is electrophoresis not used?
    a. Separation of proteins

b. Separation of nucleic acids
c. Separation of Lipids
d. Separation of amino acids

16. The most important non-covalent bond in driving proteins to fold into compact structures in water is-
a. hydrogen bonds
b. hydrophobic bonds
c. vanderwall interactions
d. ionic bonds

17. The causative agent of mad cow disease in cattle is:
a. rogue proteins
b. prions
c. infectious distorted proteins
d. all above

18. In electrophoresis, DNA will migrate towards-
a. cathode or positive electrode
b. anode or negative electrode
c. cathode or negative electrode
d. anode or positive electrode

19. pH at which a protein has a neutral charge ;loss or gain of protons in a pH gradient is _____
a. isoelectric focusing
b. electrophoresis
c. SDS-PAGE
d. isoelectric point

20. Which of the following biomolecules displays maximum diversity in function-
a. Protein
b. Lipids
c. Carbohydrates
d. Nucleic acids

ANSWER KEY

S. No.	Answer	S. NO.	Answer
1	b	11	c
2	d	12	a
3	b	13	d
4	c	14	b
5	b	15	c
6	b	16	d
7	a	17	d
8	c	18	d
9	d	19	d
10	b	20	a

5.2.2. ASSERTION REASON QUESTIONS

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

1. Assertion- In sickle cell haemoglobin, valine substitutes glutamic acid at 6th position in beta chain.
Reason: Sequence of amino acid makes a protein unique in function.

2. Assertion: Electrophoretic mobility of sickle cell haemoglobin is lesser than the normal haemoglobin.
Reason: Sickle cell haemoglobin has less oxygen carrying capacity.

3. Assertion: Isoelectric focusing separates the proteins on the basis of their pI values.
Reason: At pI value, a protein comes to rest.

4. Assertion: The major attraction of mass spectrometer is that as little as picomoles of a protein sample can be analysed.
Reason: Mass spectrometer analyse z/m ratio of protein ions.

5. Assertion: Native subtilisin loses its activity in presence of bleach.
Reason: To maintain its activity methionine 222 is substituted with cysteine 222.

ANSWER KEY

Q. No.	1	2	3	4	5
Ans.	A	B	A	C	C

5.2.3. VERY SHORT ANSWER QUESTIONS

1) In a variant of chymotrypsin, Asp102 is replaced by Glu102. Do you expect the enzyme to retain activity? Justify your answer.

Ans. Yes, I expect that the enzyme will retain its activity. Because both Asp102 and Glu102 are negatively charged amino acids and their nature is exactly same (acidic).

2) What are BCAA? Name any two BCAA. State any two functions of BCAA.

Ans. BCAAs are Branched Chain Amino Acids.
BCAA: ile, leu,val, lys, trp.

They- • are essential for the biosynthesis of muscle protein • help in increasing the bio-availability of high complex carbohydrates • reduce muscle breakdown and act as an energy source before and after exercise. Hence while maintaining exercise performance and delaying exhaustion BCAAs are very important for muscle growth.

3) Write any two properties which can be improved through protein engineering.

Ans.
- Properties for thermal and pH stability
- solvent tolerance and solubility
- catalytic potency etc.

4) Name the amino acids involved in the catalytic triad that regulates charge-relay system in the enzyme Chymotrypsin?

Ans. His-57, Asp-102 and Ser-195

5) An enzyme X is used to remove stains from fabrics. Mala added bleach and a detergent that contained enzyme X to wash her white school uniform. However, she did not get the desired result. Identify the enzyme X and provide an explanation for the inefficiency of the detergent that contains X. Suggest a solution to her problem giving proper explanation

Ans. X is Subtilisin. The native enzyme subtilisin is easily inactivated by bleach (up to 90%). Solution to the problem is to use the detergent that contains Subtilisin that is modified by Site directed mutagenesis which is not affected by bleach.

5.2.4. SHORT ANSWER QUESTIONS

1.What is in-situ activation? How does the charge-relay system operate in the enzyme Chymotrypsin?

Ans. In Situ Activation means activation of zymogens at their site of activity in the presence of their biological target by alteration in its shape.

Due to constellation of three amino acids because of unique folding of chymotrypsin, the asp102 is able to hydrogen bond with the adjacent his57 by borrowing a hydrogen ion. The his57 in turn attracts a hydrogen ion from the adjacent ser195 which allows its negatively charged oxygen anion to be able to make a nucleophilic attack on the peptide bond of the substrate.

1.A given protein with a molecular weight of 20,000 Daltons containing 5,4,3,2, and 1 charge, is subjected to mass spectrometry. Find the sequence of protein ions detected by the mass spectrometer.

Ans. $m/z = M + nH^+/n$ (Formula)

$m/z = 20,005/5 = 4001$

$m/z = 20,004/4 = 5001$

$m/z = 20,003/3 = 6668$

$m/z = 20,002/2 = 10,001$

$m/z = 20,001/1 = 20,001$

Sequence— Mass spectrometer detects protein ions at m/z = 4001, 5001, 6668, 10001 and 20,001 respectively.

2.Thalassaemic patients lack either alpha or beta subunit(s) of haemoglobin leading to impaired oxygen-binding capacity by their erythrocytes. How can the missing subunit be determined?

Ans. Normal and thalassaemic erythrocytes are obtained and their lysates are analysed via Protein fingerprinting/2-D gel electrophoresis/MALDI-TOF/SDS-PAGE so as to identify if α or β chain is absent.

3.Which functional property of whey protein is exploited in the following food systems:

(i) Eggless cakes
(ii) Soups
(iii) Coffee whiteners

Also, state their mode of action.

Ans.

Food systems	Functional Property	Mode of action
(i) Eggless cakes	Whipping/ foaming	Forms Stable film
(ii) Soups	Viscosity	Thickening/ water binding
(iii) Coffee whiteners	Emulsification	Formation and stabilization of fat emulsions

5.2.5. LONG ANSWER QUESTIONS

1) How was it be proved that sickle cell anaemia results from an amino acid substitution in Haemoglobin? Elaborate it.

Ans. One of the first attempts to study the molecular basis of sickle cell anaemia was to compare the electrophoretic mobility of normal (Hb) and sickle cell haemoglobin (scHb). On finding that Hb moved faster than scHb, Linus Pauling predicted that the latter differed in a charged amino acid. This was confirmed by V. M. Ingram in 1957 who pioneered a useful technique called protein finger printing in the famous Laboratory of Molecular Biology (LMB) at Cambridge, UK.

Protein Finger printing- Peptide Mapping
Each protein has a unique peptide map (2-D map) and hence serves as a fingerprint for the protein.

a) Pure Hb and scHb are taken separately into test tubes.
b) The Hb and scHb are digested with the proteolytic enzyme trypsin which cleaves the protein after basic amino acid residues Arg and Lys.
c) 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. Highly charged peptides will migrate more towards the anode/cathode.

d) The paper strips are dried, attached to larger squares of Whatman paper and chromatographed at right angles to the electrophoretic direction using a solvent system Butanol: Water:Acetic acid. In such a system peptides will separate based on their partition coefficient between the solvent and paper which is dependant on the relative hydrophobicity of the peptides. More hydrophobic peptides will move with the solvent to longer distances.

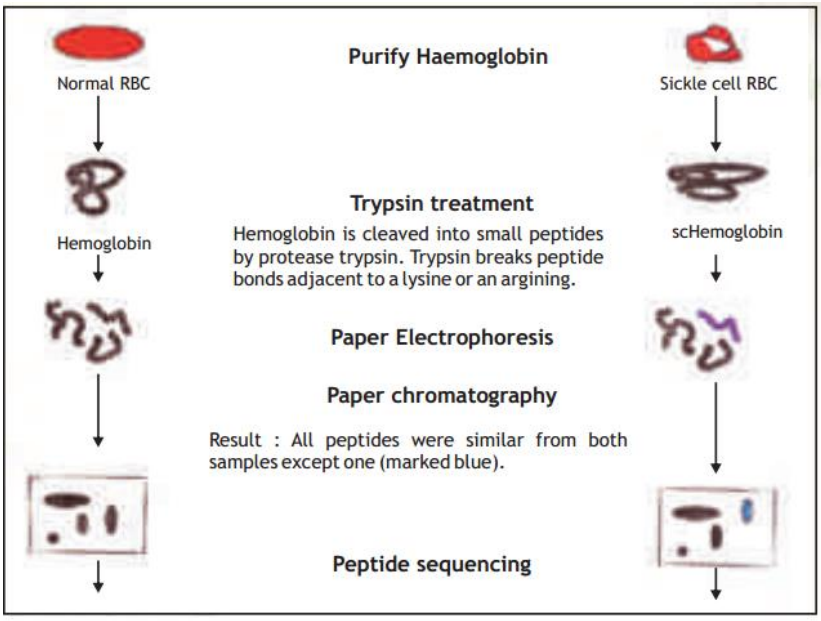


Fig. 1. Protein Finger Printing- Peptide Mapping

e) The chromatograms are dried and stained with a suitable visualisation reagent like Ninhydrin wherein peptide containing regions appear as orange yellow spots.

f) The peptide map for Hb and scHb are compared and it was found that one peptide was differently placed in the scHb map.

g) On examining this peptide and determining its amino acid sequence, Ingram found that it had a valine substitution for glutamic acid in the peptide.

The single substitution of valine for glutamic acid (at the 6th position of the beta chain) dramatically changes the structure of scHb making it form fibres within the RBC resulting in the deformation of the cell (sickling). Since the disease was due to a molecular alteration the term molecular disease was applied.

2) (a) Illustrate the important parts of a mass spectrometer with the help of a suitable diagram. (b) Explain how proteins are volatilised as well as analysed by a mass spectrometer. (c) What is the major attraction for using this technique as a characterization tool for proteins.

Ans. (a) Mass Spectrometer

(b) The protein molecules can be vaporized by using the method called matrix assisted laser desorption ionisation where a pulsed laser beam is directed onto sample suspended in a matrix.

The protein molecules can be analysed by separating and directing the charged ions by electrostatic lenses from ionisation source to the mass analyser.

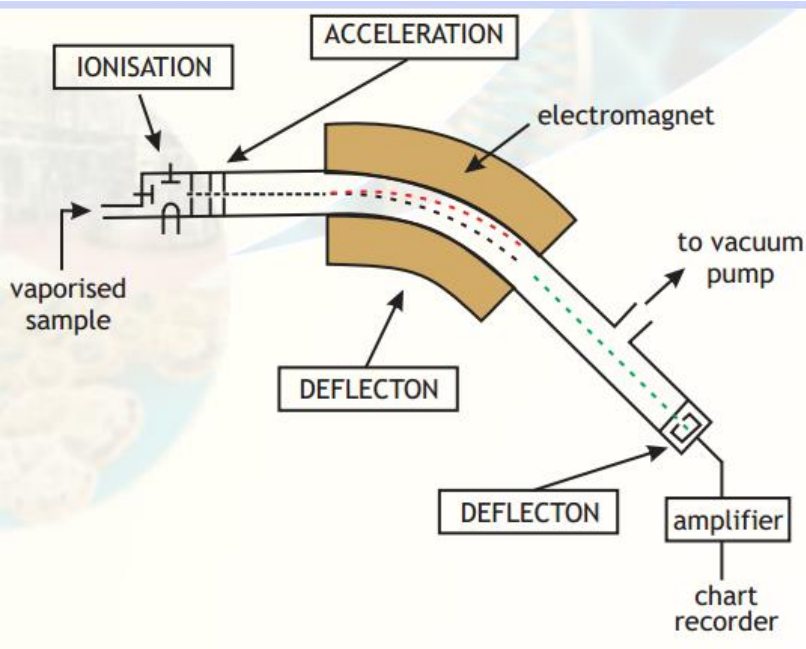


Fig. 10. An outline of a mass spectrometer.

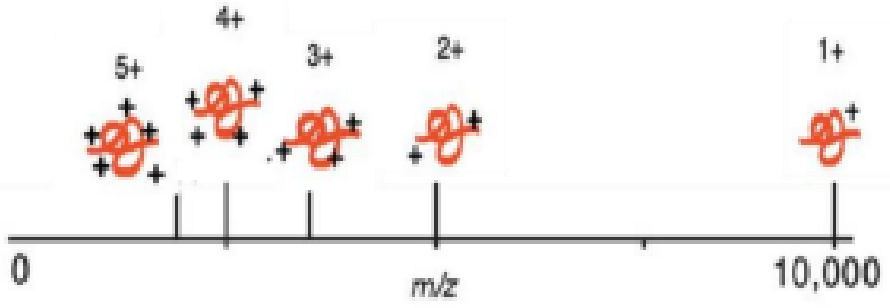
(c) It can provide information about molecular weight of unknown molecules/ structural information/Pico moles of protein samples can be analysed too.

3) Explain any five protein-based products.

Ans. (i) Blood products and vaccines e.g. Factor IX for treating haemophilia B
(ii) Therapeutic antibodies and enzymes e.g. Monoclonal antibodies OKT3 for preventing graft rejection.
(iii) Therapeutic hormones and growth factors e.g. Insulin to treat diabetes.
(iv) Regulatory factors e.eg. Interferons for antiviral properties.
(v) Analytical applications e.g. Horse radish peroxidase for ELISA.
(vi) Industrial enzymes e.g. Papain for meat tenderization.
(vii) Functional non-catalytic proteins e.g. Kappa casein for milk protein stabilization.
(viii) Nutraceutical proteins e.g. Infant food formulation to provide adequate nutrition for infant.

5.2.6. CASE BASED QUESTIONS

1) Mass Spectrometry Mass spectrometry (MS) has emerged as an important tool in biotechnology. It is extremely useful in obtaining protein structural information such as peptide mass or amino acid sequences. The molecular ions are generated either by a loss or gain of a charge (e.g. electron ejection, protonation or deprotonation). After the ions are formed, they can be separated according to their m/z ratio and finally detected. A protein with a molecular weight of 10,000 dalton generates five different peaks with the ions containing 5, 4, 3, 2, and 1 charges, respectively, as shown below.



(a) What happens if there is a loss of charge from a biomolecule?
(b) Mass spectrometry is an analytical tool. Justify the statement.
(c) Calculate the m/z ratio each for protein ions containing 5, 4, 3 and 2 charges.

OR

(c) A protein has a molecular weight of 20,000 Daltons and it forms two protein ions containing 6 and 7 charges, What will be its mass/charge ratio?

Ans. (a) The molecular ions are generated either by a loss or gain of a charge (e.g. electron ejection, protonation or deprotonation)

(b) Mass spectrometry is used in- (i) Obtaining protein structural information such as peptide mass or amino acid sequence (ii) Identifying the type and location of amino acid modification within proteins. (any one)

(d) $m/z = (M + nH^+)/n+$

For n=5, m/z = 10,000+5/5 = 2001

For n=4, m/z = 10,000+4/4 = 2501

For n=3, m/z = 10,000+3/3 = 3334.3

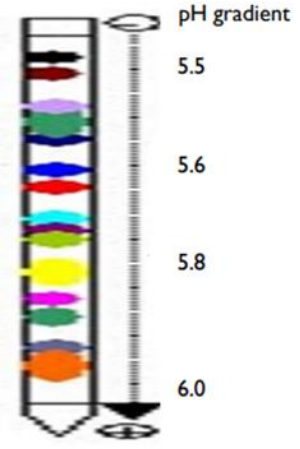
For n=2, m/z = 10,000+2/2 = 5001

OR

$m/z = (M + nH^+)/n+$

For n=6, m/z = 20,000+6/6 = 3334.33

For n=7, m/z = 20,000+7/7 = 2858.14



2) Set up given is an IEF gel for the separation of four proteins A, B, C and D obtained in a cell extract.

The pI of the proteins A to D is 5.5, 6, 7 and 8 respectively. Study the set up and answer the questions that follow:

pH gradient 5.5, 5.6, 5.8, 6.0

(5.5 to 6.0)

(a) Which proteins A to D can be separated using the above setup?

(b) What change in the above set up is required in order to separate all the proteins?

(c) In electrophoresis the protein will move until

1. its pH is greater than PI
2. its pH is smaller than PI
3. its pH is equal to PI
4. PI is greater than pH

(d) At certain pH environments the isoelectric point affects the-

1. solubility of molecule
2. solubility of solvent
3. temperature
4. density of molecule

Ans. (a) Protein samples A and B will get separated using this set up.

(b) Using ampholytes with broader range covering pH value ranging from 3 to 11, we will be able to isolate all the four proteins.

(c) its pH is equal to PI

(d) solubility of molecule

UNIT-5

PROTEIN AND GENE MANIPULATION

CHAPTER-5.3

GENOMICS, PROTEOMICS & BIOINFORMATICS

5.3.1. Genomics (Genome+omics)

In 1920, the term "Genome" was coined by H. Winkler to denote the complete set of chromosomal and extra-chromosomal genes present in an organism.

In 1987, the term "Genomics" was coined by Thomas H. Roderick. At that time, this term was taken to mean sequencing and mapping the genome to analyze its structure and organisation.

Today, **genomics** includes:-

a) sequencing of genomes
b) determination of complete set of proteins encoded by the concerned genome
c) functioning of genes and metabolic pathways in the organism.

Thus, **genomics** not only deals with the determination of the genetic information present in an organism, but also with understanding the mechanism by which this information is used by the organism.

5.3.1.1. Branches of Genomics

The discipline of **genomics** is often divided into the following two domains:-

a) Structural Genomics
b) Functional Genomics

a. Structural Genomics: deals with the determination of the complete sequence of genome as well as the complete set of proteins produced by the concerned organism.

b. Functional Genomics: deals with the study of functioning of genes and metabolic pathways, i.e., the gene expression pattern of the organism.

5.3.2. Bioinformatics (Biology + Information Technology)

The information generated in genomics is enormous.
Management and interpretation of this information requires the use of computers.

Bioinformatics is an emerging field concerned with the development and application of computer hardware and software to the acquisition, storage, analysis and visualisation of biological information.

5.3.3. Proteomics (Proteome+omics)

Proteomics is the study of gene products encoded by a genome.

The major areas of study are as follows:-

a) identification of the genes which are expressed
b) their time of expression
c) the type and extent of any post-translational modification of the gene product, i.e., protein
d) the function of the encoded protein & its location in various cellular compartments.

Table: A brief description of various terms

Term	Meaning
Genome	The complete set of genetic information
Genomics	Genome structure and function
Structural Genomics	Structure of the genome and the proteins it encodes
Functional Genomics	Gene expression, regulation and phenotype production
Proteome	The complete set of proteins encoded by the genome
Proteomics	Determination of the proteome
Bioinformatics	Management and interpretation of data generated by genomics

5.3.4. Gene Prediction and Counting

5.3.4.1. Gene Prediction

After a genome sequence has been obtained and checked for accuracy, the next task is to find all the protein coding genes, i.e., Gene Prediction.

- Gene prediction is the first step in **annotation.**
- **Annotation:** Annotation is a process that identifies genes, their regulatory sequences and their function(s).
- Annotation also identifies non-protein coding genes, including those that code for r-RNA, t-RNA and small nuclear RNAs.
- Annotation also identifies mobile genetic elements and repetitive sequences present in the genome.

- Locating protein-coding genes is done by inspecting the sequence using a computer software or by eye.
- Genes have several identifying features:-

a) In prokaryotes-

(i) Protein-coding genes are composed of open reading frames (ORFs).

ORF: An **ORF** has a series of codons that specify an amino acid sequence.

They begin with an initiation codon (ATG) and end with a termination codon (TAA, TAG, or TGA).

ORFs are identified usually by some computer software (ORF scanning software).

(ii) Additional computer programmes which can identify genes in prokaryotes are GENMARK, Glimmer, etc.

a) In eukaryotes- genes have several features that make their searching difficult.

(i) Genes in eukaryotes have a pattern of exons (coding regions) alternated with introns (noncoding regions) i.e., Split Genes. As a result, these genes are not organized as continuous ORFs.

(ii) Genes in eukaryotes are often widely spaced, increasing the chances of finding false genes.

- That's why, for making searching possible in eukaryotes, several sophisticated computer softwares

have been developed for gene prediction, e.g., GENIE,
GENESCAN, GRAIL, GeneFinder, HMM Gene, etc.

- But none of the above programmes is 100% accurate
  at identifying genes.

5.3.4.2. Gene Counting

- ven if we know where the genes are in the genome, it is not entirely clear how to count them.
- Due to existence of **over-lapping genes** and **splice variants** it is difficult to define the parts of the DNA that should be regarded as the same or several different genes.

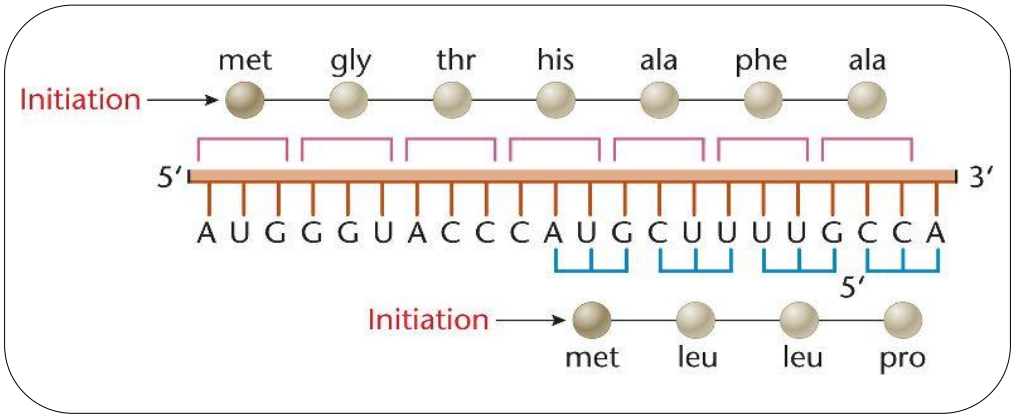


Fig: Over-lapping Genes

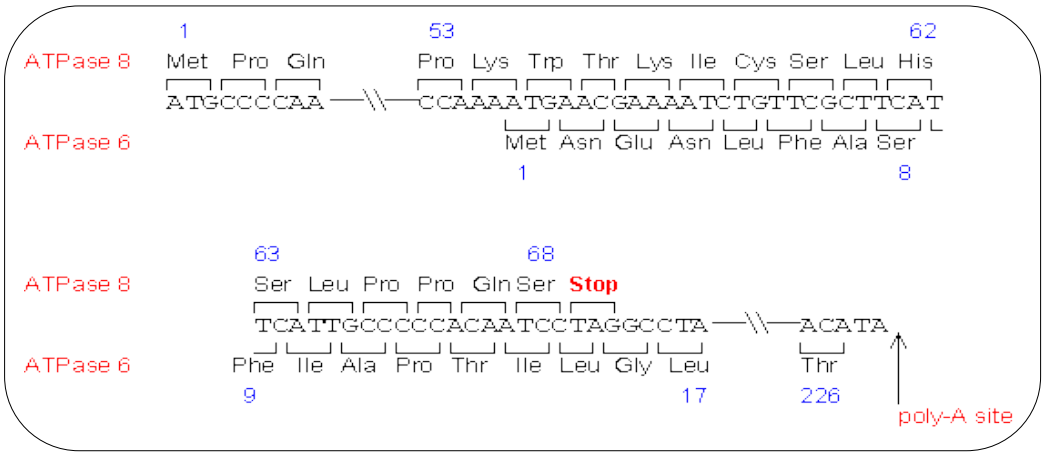


Fig: Over-lapping Genes

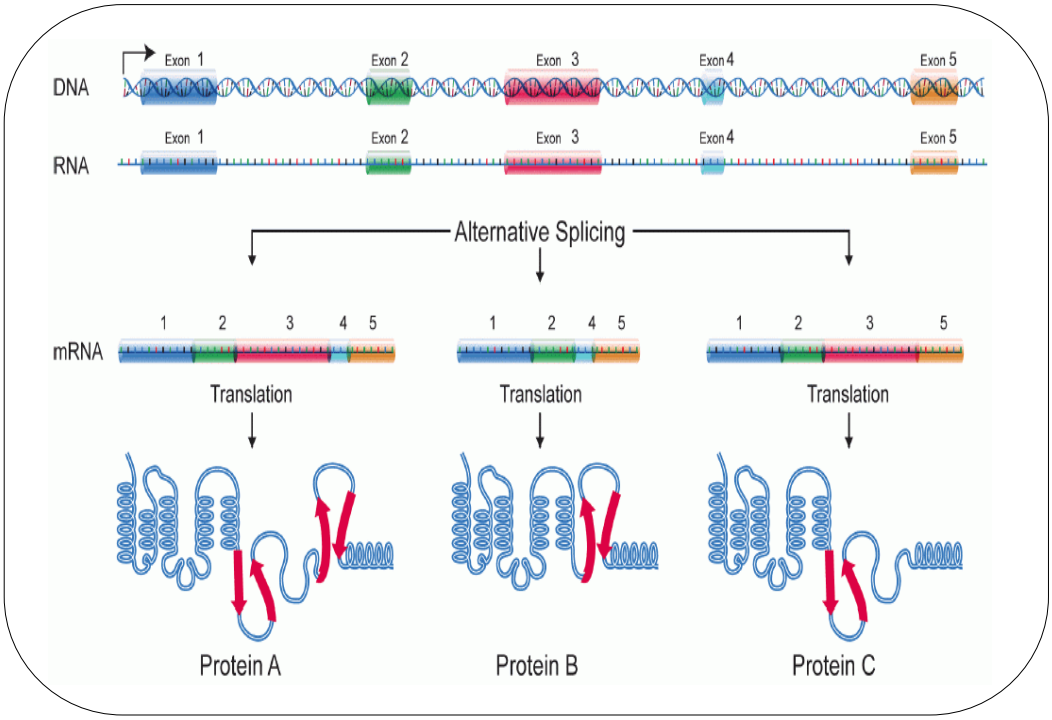


Fig: Splice Variants

- Nevertheless, for practical purposes (allowing for some "experimental error") we can count number of genes present in the genome of an organism.

Organism	Genome size (bp)	Number of predicted genes
Escherichia coli	5,00,000	5,000
Saccharomyces cerevisiae	12,068,000	6,340
Caenorhabditis elegans	100,000,000	19,000

Drosophila melanogaster	175,000,000 – 196,000,000	13,600
Arabidopsis thaliana	157,000,000	25,498
Homo sapiens	3,000,000,000	30,000-35,000

Table: Showing Genome size and the predicted number of genes

5.3.5. Genome Similarity

One of the surprising findings from the analysis of genome sequences of different organisms is that the genomes of organisms differing remarkably in appearance may be quite similar.

1) Human and mouse are so much different in their appearance but these two organisms share about **97.5 %** of their DNA sequences **that perform genetic functions.**

   It has now been concluded that humans and mice diverged from a common ancestor some 100 million years ago. During this period, their functional DNA has diverged only to a limited extent, while their non-coding DNAs have diverged to a much greater extent.

2) Similarly, it is estimated that human and chimpanzee genomes differ for only 1 to 3% of their DNA sequence.

   Because of this similarity, evolutionists have viewed the chimpanzee as “our closest living relative”.

5.3.6. SNPs and Comparative Genomics

SNPs (Single Nucleotide Polymorphisms) are single base positions in genomic DNA at which different nucleotides occur in different individuals of a population. Each nucleotide at such a position denotes an allele of the SNP. It is estimated that 90% of sequence variation in humans is due to SNPs.Human genome is estimated to contain 3-17 million SNPs. Of these 5% of the SNPs are expected to occur within genes.
Thus each gene may be expected to contain ~6 SNPs.

Thus SNPs provide a molecular marker that occurs in genome at a very high density.

The SNPs can, therefore, be used to map genes involved in human diseases.

By using SNPs as markers, each gene in human genome can be mapped.
Further, it should be possible to identify all the genes involved in human diseases.

The variations in genetic sequences of different individuals (due to SNPs) are thought to be involved in:

a) Disease susceptibility
b) Response to environmental factors
c) Drug response
d) Normal development and aging

5.3.6.1. Pharmacogenomics

The field of study that is concerned with the effect of genetic variation on disease susceptibility and drug response is known as pharmacogenomics.

Pharmacogenomics is expected to develop individualized treatments that will be safer as well as more effective.

The genetic differences among individuals may affect drug response in the following ways:

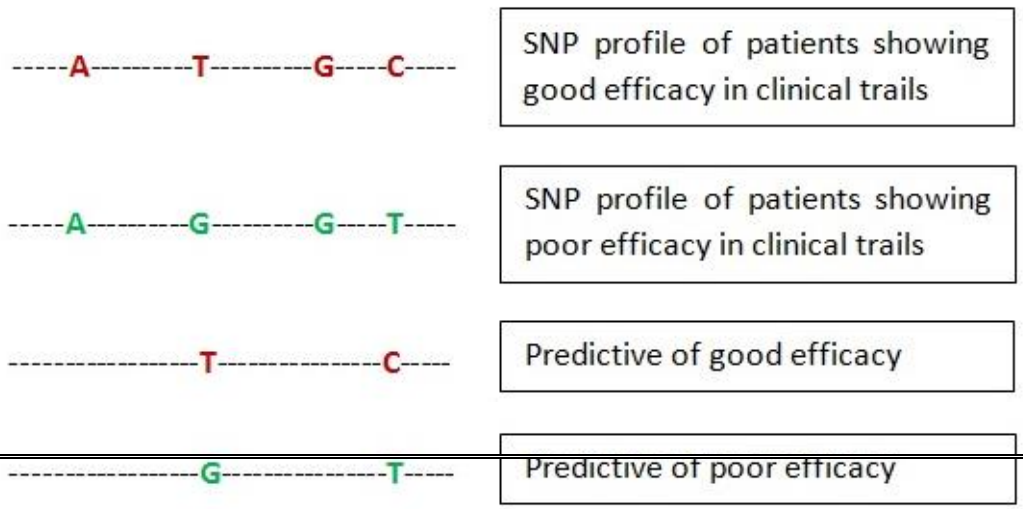
a) They may affect the metabolism of the drug itself
b) They may influence the action of the drug on its target molecule.

The drug response is likely to be affected by several genes concerned with drug metabolism, drug transport and production of drug targets.

Efforts are being made to use SNPs to map hundreds or thousands of genes that have an effect on the safety and efficacy of various drug treatments.

Before prescribing treatment, physicians can use patients DNA sample to determine the pattern of SNP genotype profile and from that they can predict how the patients are likely to respond to a given drug.

Fig: How to predict response to drugs using SNP profile



5.3.6.2. 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

5.3.7. Functional Genomics

Functional genomics dissects the emerging knowledge about genomes to understand the gene and their product functions and interactions. Two exciting new developments are now enabling scientists to get a wealth of clues to this complicated story:-

a) Fluorescence in-situ hybridization
b) Microarray Technology

5.3.7.1. Fluorescence in-situ hybridization

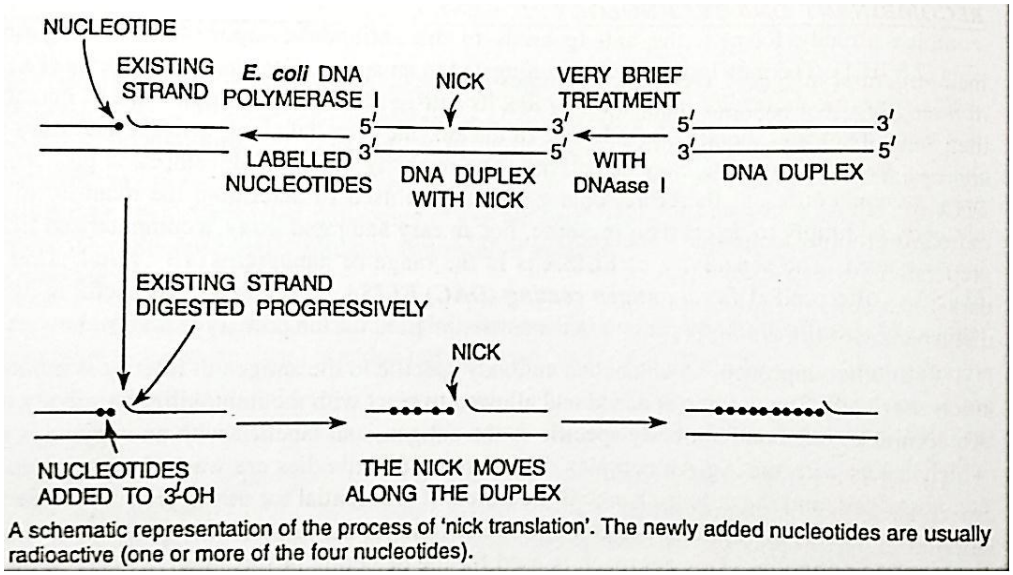
It is a technique which is used to detect and localize the presence or absence of specific DNA sequences (genes) on chromosomes within cells or tissue sections fixed on a glass slide/solid support.

This technique uses fluorescent probes that bind to only those regions of the chromosome with a high degree of sequence complementarity.

Fluorescence microscopy can be used to find out where the fluorescent probe is bound to the chromosome by detecting fluorescence.
The fluorescent probe used in FISH can be produced via the process of Nick Translation which is nothing but a method of DNA labelling (radioactive or fluorescent labelling).

Nick translation can be used for preparation of radioactively or fluorescently labelled probes to be used in various hybridization experiments.

Fig:



A schematic representation of the process of 'nick translation'. The newly added nucleotides are usually radioactive (one or more of the four nucleotides).

Nick Translation

5.3.7.1.1. Process of FISH- Steps of the process

a. Preparation of probe (fluorescently labelled): the base sequence of probe must be complementary to the sequence of the gene to be detected & located.

b. Fixation of cells or tissues on glass slide followed by their permeabilization: Fixatives are used which preserve cell morphology while concomitantly permeabilizing all cells for the labelled oligonucleotide probe.

c. Denaturation of DNA inside fixed cells or tissue by using formamide at 42ºC.

d. Allow the fluorescent probe to hybridize with the fixed cells or tissue.

e. Washing to remove unbound probe.

Observe under fluorescence microscope to detect the location of the bound probe by detecting the fluorescence associated with probe.

5.3.7.1.2. Application of FISH

In detecting the severity of a disease "chronic myelogenous leukemia" (CML): cancer of white blood cells.

CML is associated with a reciprocal translocation between Ch9 and Ch22.

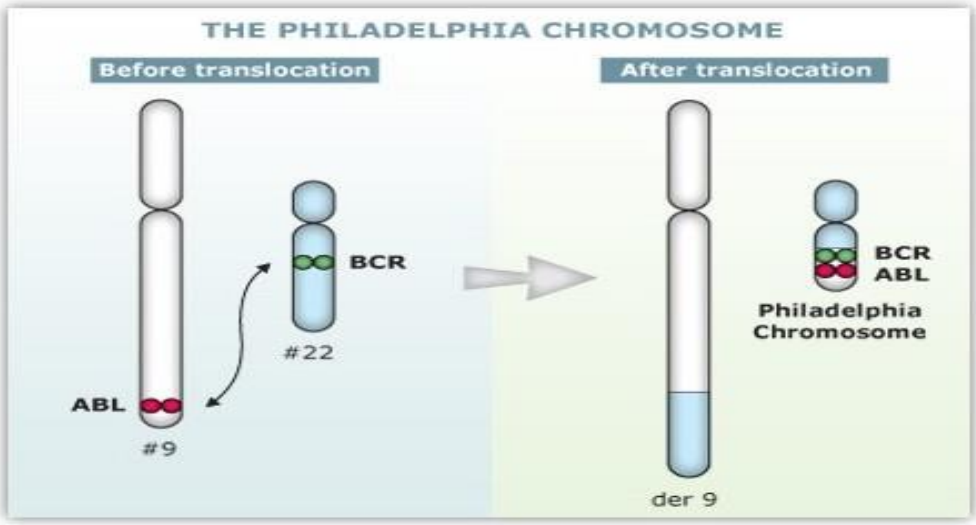


Fig: Reciprocal Translocation between Ch9 and Ch22 to give rise to Philadelphia Chromosome

From the DNA library, it was possible to pick up clones carrying the genes involved in CML (*abl* and *bcr*).

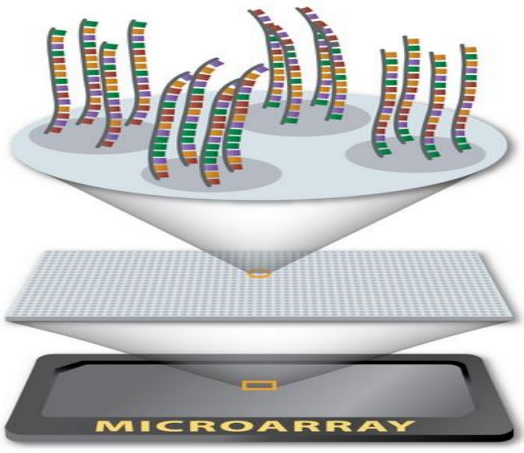
Using nick translation, it was possible to prepare differently coloured fluorescent probes for both of these genes (*abl* and *bcr*).

Suppose that the probe for *abl* is tagged with red and the probe for *bcr* is tagged with green fluorescent tag, the process of FISH can be followed as below:-

1. Fixing of blood lymphocytic cells on glass slide & their permeabilization.
2. Denaturation of DNA inside fixed cells.
3. Allow both the probes to hybridize simultaneously with the cells.
4. Washing to remove unbound probe.
5. Visualisation under fluorescence microscope.
6. Counting of cells showing yellow fluorescence, due to overlapping of red and green fluorescence, to have an idea of disease severity.

5.3.7.2. Microarray Technology

An array is an orderly arrangement of data.



A DNA microarray consists of large number of single stranded DNA molecules/fragments, representing different genes of an organism,

spotted in a systematic order at fixed locations as microdots on a solid substrate, usually a glass slide or silicon wafer.

Each spot on a microarray contains multiple identical strands of DNA (representing a gene).

The DNA sequence on each spot is unique and represents one gene.

Thousands of spots are arrayed in orderly rows and columns on a solid surface (usually glass).

The precise location and sequence of each spot is recorded in a computer database.

5.3.7.2.1. Types of DNA Microarray

5.3.7.2.2.

Spotted DNA Microarray	**Oligonucleotide Microarray (DNA chips/Biochips)**
DNA sequences representing different genes of an organism are spotted onto the slide.	Different oligonucleotides representing different genes of an organism are synthesized directly onto a silicon wafer using the technique of photolithography.
Gene/DNA sequences can be obtained from genomic library of the organism.	

Comparative cDNA Hybridization Microarray

To perform a comparative cDNA microarray analysis:-

1.mRNA molecules are typically collected from both an experimental sample and a reference sample. For example, the reference sample could be collected from a healthy individual, and the experimental sample could be collected from an individual with a disease like cancer.

2.The two mRNA samples are then converted into complementary DNA (cDNA), and each sample is labelled with a fluorescent probe of a different colour. For instance, the experimental cDNA sample may be labelled with a red fluorescent dye, whereas the reference cDNA may be labelled with a green fluorescent dye.

3.The two samples are then mixed together and allowed to bind to the microarray slide.

4.The process in which the cDNA molecules bind to the DNA probes on the slide is called hybridization.

5.Following hybridization, the microarray is scanned to measure the expression of each gene printed on the slide.

6.If the expression of a particular gene is higher in the experimental sample than in the reference sample, then the corresponding spot on the microarray appears red.

7.In contrast, if the expression in the experimental sample is lower than in the reference sample, then the spot appears green.

8.Finally, if there is equal expression in the two samples, then the spot appears yellow.

9.The data gathered through microarrays can be used to create gene expression profiles, which show simultaneous changes in the expression of many genes in response to a particular condition or treatment.

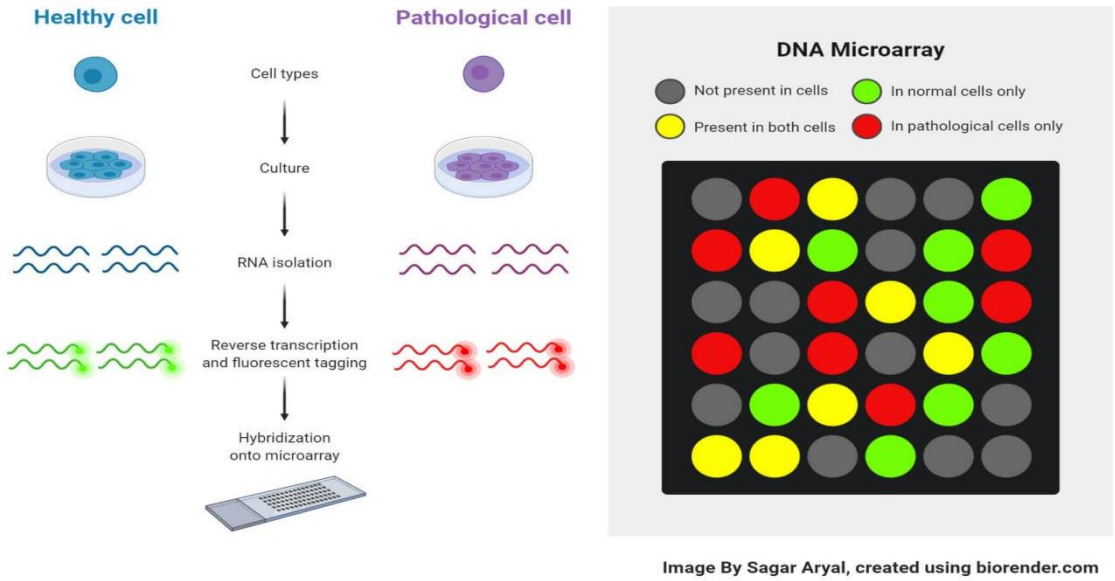


Fig: Comparative cDNA microarray hybridization analysis

5.3.7.2.3. Applications of Microarray Technology

Can be used to reveal the identity of genes being expressed in a cell or tissue of an organism at a given point of time or in a particular environmental condition, i.e., expression profiling.

Gene expression profiling is the measurement of the activity (the *expression*) of thousands of genes at once, to create a global picture of cellular function.

Microarray technology has been used to study the following:

1. Tissue specific genes
2. Regulatory gene defects in a disease
3. Cellular responses to environment
4. Cell cycle variations

5.3.8. Proteomics

Proteome: the complete set of proteins expressed during a cell's entire lifetime.

Proteomics: It is the large scale study of the proteome of living organisms.

Study areas under Proteomics:-

1) protein-protein interaction studies
2) protein function
3) protein localisation
4) protein expression

5.3.8.1. Branches of Proteomics

Structural Proteomics: Mapping out the 3D structure and nature of protein complexes present specifically in a particular cell/organelle.

Aim of Structural Proteomics:

1) To identify all the proteins, present in a complex
2) To characterize all protein-protein interactions occurring among these proteins.

Functional Proteomics: Use of proteomics techniques to analyse characteristics of molecular protein-networks involved in a living cell.

One of the recent successes of this field is the identification and analysis of molecular protein-network involved in nuclear pore complex (NPC) in yeast. This success helps to understand the translocation of molecules from nucleus to the cytoplasm and *vice-versa.*

Expression Proteomics: Quantitative study of protein expression between samples differing by some variable.

The pattern of expression of the complete proteome or of its part (sub-proteome) between samples can be compared.

Expression proteomics is quite useful in **expression profiling.**

5.3.8.2. Proteomics Areas & phenomenon of their interest

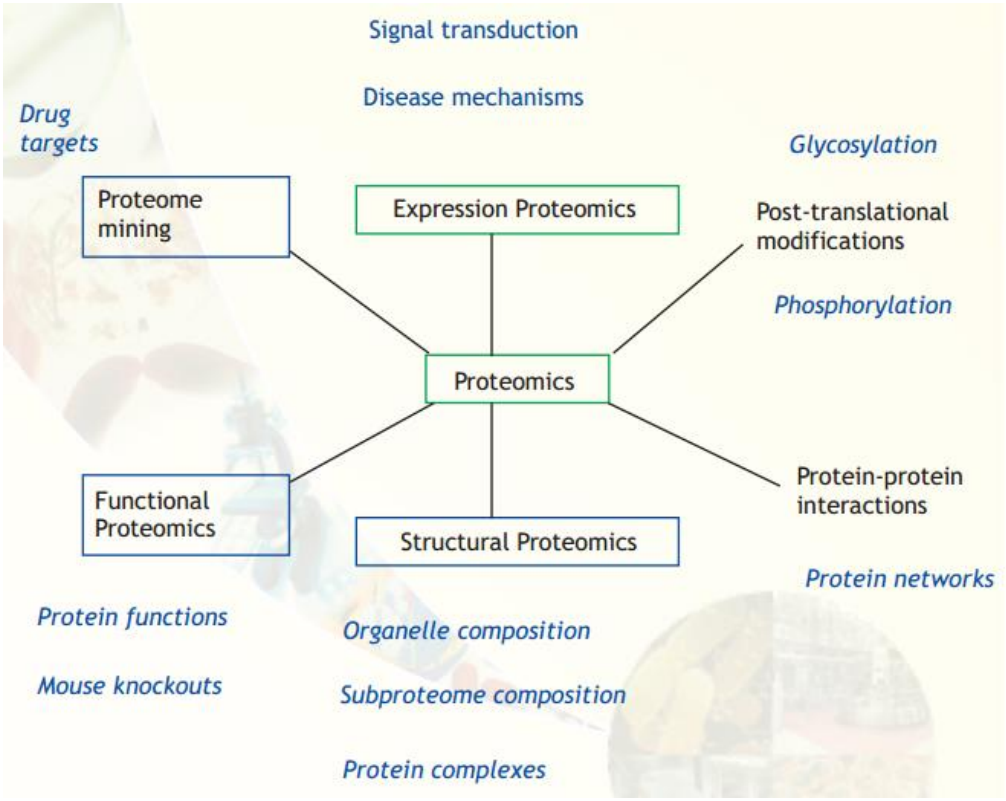


Fig: Types of proteomics & scientific knowledge that can be gained from them

5.3.8.3. No. of Genes v/s No. of Proteins

- Generally, it is assumed that one gene codes for one protein.
- But, actually, the relationship between the no. of genes and no. of proteins appears to be non-linear.
- Usually, each gene gives rise to multiple protein forms having different structural and functional properties.
- In other words, the number of proteins easily outnumber the number of genes.

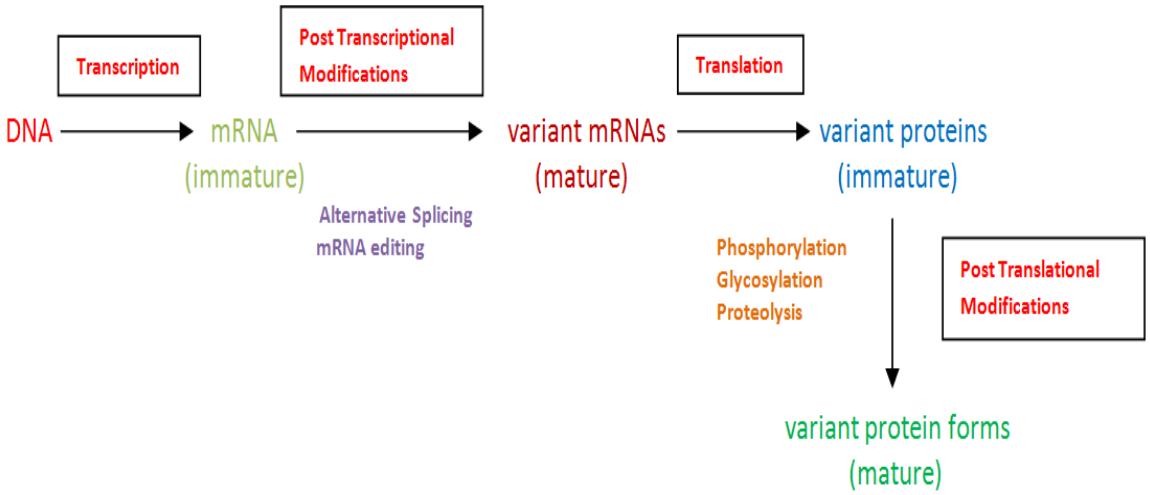


Fig: Processes through which genes can give rise to multiple protein products with differing functions.

5.3.9. Bioinformatics

Bioinformatics = Biology + Information technology

- Bioinformatics is the science concerned with the development and application of computer hardware and software to the acquisition, storage, analysis and visualisation of biological information.

5.3.9.1. Bioinformatics: Chief Components

- The development of **database** for an efficient storage, access and management of the large body/amount of various biological data.

- The development of **new algorithms** and **statistical methods** for analysis and interpretation of the various biological data.

- The application of the above tools for the **analysis and interpretation** of the various biological data, including nucleotide sequences, amino acid sequences, etc.

5.3.9.2. Databases and Retrieval Tools

- **Database:** A database is a vast collection of data pertaining to a specific topic, e.g., nucleotide sequence, protein sequence, etc., in an electronic environment.

- **Database Retrieval Tools:** The utilization of various databases requires the use of suitable search engines and analysis tools. These tools are often called *database retrieval/mining tools* and the process of database utilization is known as *database mining.*

5.3.9.3. Bioinformatics Organizations

- There are three public domain bioinformatics facilities:

1) National Centre for Biotechnology Information (NCBI) located at National Institute of Health (NIH), USA
2) European Bioinformatics Institute (EBI), U.K.
3) GenomeNet (Japanese Bioinformatics Service), Japan

These organizations develop databases as well as appropriate retrieval tools.

The computational tools and databases are essential to the management and utilization of the rapidly expanding volume of biological data.

5.3.9.4. Databases:

Databases are mainly of two types-

(1) Nucleotide sequence Databases

(2) Protein databases

Database	Information available	Source
GenBank	Genomic DNA sequences	NCBI, USA
EMBL Nucleotide Sequence Database	Nucleotide sequences (DNA & RNA)	EBI, UK
DDBJ (DNA Data Bank of Japan)	Nucleotide sequences	GenomeNet, Japan
dbEST	EST Sequences from GenBank, EMBL and DDBJ databases	NCBI, USA; EBI, UK
HomoloGene	Homologous genes in human, mouse, rat, zebrafish, cow, etc.	NCBI, USA
UniGene	EST sequences related to one gene grouped into sets called “cluster”	NCBI, USA

5.3.9.4.1. Nucleotide Sequence Databases

5.3.9.4.2. Protein Databases

Database	Information available	Source
PDB (Protein Data Bank)	Sequences of those proteins whose 3D structures are known	NCBI, USA; EBI, UK
PIR (Protein information resource)	Protein sequences	NCBI, USA
RefSeq	mRNAs and proteins of human, mouse and rat	NCBI, USA
SWISS-PROT	Protein sequences	NCBI, USA; EBI, UK
PALI Database	Phylogenetic analysis and alignment of proteins	IISc, Bangalore, India
UniProtKB	Annotated protein sequences	NCBI, USA; EBI, UK

5.3.9.5 Database Retrieval Tools

Retrieval Tools	**Function provided**
BLAST (Basic Local Alignment Search Tool) (NCBI, USA)	A group of tools used to analyze sequence information and detect homologous sequences
ENTREZ (NCBI, USA)	Used to access literature (abstracts), sequence and structure databases
LOCUS LINK (NCBI, USA)	Accessing information on homologous genes, i.e., genes of different species that are similar due to common ancestry
TAXONOMY BROWSER(NCBI, USA)	Taxonomic classification of various species as well as genetic information

5.3.9.6 BLAST (Basic Local Alignment Search Tool)

- BLAST is a family of user-friendly sequence similarity search tools on the web.
- The BLAST server is supported through NCBI, USA.
- This tool is designed to identify potential homologues for a given sequence.
- It can analyze both DNA and protein sequences.
- Identification of homologues allows the prediction of probable functions of the concerned gene/protein.

5.3.9.6.1. Types of BLAST Programmes

- *BLASTp* : It compares the submitted protein sequence against a protein database.
- *BLASTx* : It translates the submitted nucleotide sequence into amino acid sequence and compares the latter with a protein database.
- *BLASTn* : This is used to compare a nucleotide sequence with a nucleotide sequence database.
- *tBLASTn* : It converts the submitted protein sequence into nucleotide sequence and compares it with a nucleotide sequence database.

5.3.9.6.2. BLAST Comparison Strategy

- Let us suppose, researcher has generated a nucleotide (DNA/RNA) or amino acid (protein) sequence. He wishes to compare this sequence with those contained in a database with a view to identify homologous sequences.
- The BLAST search tool carries out the comparison using a set of algorithms.
- In simple terms, the logic used by BLAST programmes is as follows :

1) The sequence submitted by researcher is compared base-per-base or amino acid-per-amino acid with the database sequences.

2) A substitution scoring matrix is used by the BLAST programmes. Each match is awarded a specified score, while each mismatch is penalized by a specified negative score.

3) The sequence alignment is then assigned an overall score, which is the summation of scores assigned to each of its paired nucleotides/amino acids.

4) Top scoring alignments are ranked according to set criteria. These criteria distinguish between a similarity due to common ancestry and that due to random chance.

5) Discovered homologies or matches are further examined using information accessible through ENTREZ and other search tools.

5.3.9.7 Analysis using Bioinformatics Tools

Processing raw information: The experimentally determined sequence (raw information) is processed using bioinformatics tools into genes, the proteins encoded and their function, the regulatory sequences, and inferring phylogenetic relationships.

Detection of genes: Gene prediction can be done using computer programmes like GeneMark for bacterial genomes and GENSCAN for eukaryotes.

Proteins: Protein sequences can be inferred from the predicted genes by using simple computer programmes.

Identification of Function of a new gene: The simplest way to identify the function of a new gene is as follows :

The gene sequence is translated into the amino acid sequence of the protein it is expected to encode. This protein sequence is then compared with a protein database. A programme like *tBLASTx* will

perform both these functions. If the encoded protein is homologous to a protein in the database, it confirms the identification of the new gene. It also suggests the function of the new gene.

- **Identification of Functional Domains:** There are several bioinformatics tools for the identification of protein motifs and protein domains. Some of these tools are PRINTS, PROSITE, SMART, BLOCKS, etc.

- **Identification of regulatory sequences of genes**

- **Inferring phylogenetic relationships:** Information regarding the relationships between organisms can be obtained by aligning sequences (DNA/protein), calculating evolutionary distance and constructing phylogenetic trees.

5.3.1. MULTIPLE CHOICE QUESTIONS

1. "Even if we know where the genes are in the genome, it is difficult to exactly count them". The reason being:-

   A. Overlapping Genes
   B. Primer Excision
   C. Semi discontinuous synthesis
   D. Degeneracy of genetic code

2. In accordance with the given phylogenetic tree, which of the following organisms are expected to have highest genome similarity?

   A. B and C
   B. A and B
   C. C and E
   D. D and E

3. A single base difference in the ApoE gene is associated with?
    A. Migraine
    B. Huntington disease
    C. Cystic Fibrosis
    D. Alzheimer's disease

4. As per the studies conducted in the field of comparative genomics, how much genetic similarity exists in between the functional genome of human and mouse?
    A. 20%
    B. 80%
    C. 97.5%
    D. 99%

5. How many types of haplotypes are present in the DNA sequences shown herewith?
    A. 2
    B. 3
    C. 4
    D. 5

6. How many SNP loci are present in the aligned sequences shown herewith?
    A. 1
    B. 2
    C. 3
    D. 4
7. In CML (chronic myeloid leukemia), which of the following chromosomes undergo reciprocal translocation?
    A. Ch10- Ch22
    B. Ch9- Ch12
    C. Ch9- Ch22
    D. Ch10- Ch12
8. The full form of FISH is:-
    A. Fluorescent in situ hybridization
    B. Fluorescence in situ hybridization
    C. Filament in situ hybridization
    D. Fragment in situ hybridization

9. The main purpose of nick translation is:-
   A. To introduce fluorescent or radioactive label in the DNA
   B. To synthesize protein from nicked mRNA
   C. To reverse transcribe mRNA into cDNA
   D. To create variant forms of protein from mRNA

10. During detection of CML through FISH, which of the following fluorescent signals denote the cancerous state?
   A. abl- red signal
   B. bcr- green signal
   C. abl-bcr yellow fusion signal
   D. either a or b

11. Which of the following statements about DNA microarray is false?

A. Microarray exploits the preferential binding of complementary single stranded nucleic acids

B. A DNA microarray consist of large numbers of DNA molecules from two different organisms spotted in a systematic order on a solid substrate, usually a slide

C. Scientist can study many genes at a time using a single microarray chip

D. Microarray can be used for expression profiling of the organisms

12. In a comparative cDNA hybridization microarray experiment of normal and cancer cell using green and red fluorescently tagged cDNA molecules as probes for normal and cancer cell respectively, the microarray spot(s) showing yellow fluorescence after microarray imaging/scanning will mean which of the following :-

A. the concerned gene is being equally expressed in normal and cancer cell
B. the concerned gene is being expressed only in the normal cell
C. the concerned gene is being expressed only in the cancer cell
D. the concerned gene is not being expressed in normal as well as cancer cell

13. “Relation between number of genes and number of proteins in non-linear”, because: -

A. One gene codes for only one protein
B. Gene may not always code for protein
C. One protein coding gene usually codes for variant protein forms
D. Two genes may sometimes give rise to the same protein

14. Full form of BLAST is:-

A. Biological Local Alignment Search Tool
B. Basic Local Alignment Similarity Tool
C. Basic Local Alignment Search Tool
D. Basic Local Application search Tool

15. Taxonomy browser provides which of the following information?

A. information on the official gene names and other descriptive information about genes
B. comprehensive information on a given biological question
C. information on taxonomic classification of various species
D. information on homologous genes

16. Identify the correct match from the following:

S. No.	Database	S. No.	Information Provided
1	EMBL	a	Three dimensional structure of proteins
2	UniPro KB	b	Phylogenetic analysis and alignment of proteins
3	PALI database	c	Annotated protein sequence
4	PDB	d	Nucleotide sequence

A. 1-b, 2-d, 3-c, 4-a

B. 1-d, 2-c, 3-b, 4-a
C. 1-c, 2-a, 3-d, 4-b
D. 1-a, 2-b, 3-c, 4-d

ANSWER KEY

S. NO.	ANSWER	S. NO.	ANSWER
1	A	**9**	A
2	B	**10**	C
3	D	**11**	B
4	C	**12**	A
5	D	**13**	C
6	C	**14**	C
7	C	**15**	C
8	B	**16**	B

5.3.2. ASSERTION REASON QUESTIONS

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

11) Assertion- Cystic Fibrosis is an autosomal recessive disease.

Reason- The most common mutation responsible for this disease is a deletion of 4 bps resulting in the loss of codon no. 506, which codes for phenylalanine.

12) Assertion- Chronic myeloid leukemia is a type of cancer of skin cells.

Reason- The main cause of this disease is reciprocal translocation between Ch6 and Ch22 giving rise to Philadelphia chromosome.

13) Assertion- The proteome of a given cell is dynamic.

Reason- In addition to identification of proteins, one of the major goals of proteomics is to characterize post-translational modifications on proteins.

14) Assertion- LocusLink is a type of database retrieval tool developed by NCBI, USA.

Reason- LocusLink carries information on the official gene names and other descriptive information about genes.

15) Assertion- Genome similarity search studies are really helpful in working out the evolutionary relationship among different species/organisms.

Reason- More the genome similarity between two organisms/species, lesser they are expected to be closer phylogenetically.

ASSERTION- REASON QUESTIONS

ANSWER KEY

Q.NO.	1	2	3	4	5
ANSWER	C	D	B	A	C

5.3.3. VERY SHORT ANSWER QUESTIONS

1) Name any two diseases showing gene polymorphism with complex inheritance.

Ans. Two diseases showing gene polymorphism with complex inheritance-
- Common late-onset Alzheimer's disease
- Migraine

2) What is the role of the curator in Bio-informatics.

Ans. The curator reviews and checks newly submitted data to ensure that biological features are adequately described and the conceptual translations of any coding regions obey known rules.

3) Given below is a list of the first 06 residues of the beta helix in myoglobin from different organisms.

Position → Organism↓	1	2	3	4	5	6
Human	D	I	P	G	H	G
Chicken	D	I	A	G	H	G
Alligator	K	L	P	E	H	G
Turtle	D	L	S	A	H	G
Tuna	D	L	T	T	M	G
Carp	D	F	E	G	T	G

Based on this information, which amino acids- (a) are most conserved, and (b) are highly variable.

Ans. G amino acid is most conserved
A amino acid is most variable

4) You have the gene sequence of a protein which has a proteolytic activity. How will you establish through tools of bioinformatics that this protein-

(a) Has homologues in other organisms

(b) A database that can we used to trace the evolutionary history of this proteolytic protein?

Ans. (a) BLAST search→ Find out→ homologous sequences in other organisms by looking for gene sequence of given proteolytic enzyme.

(b) Phylogenetic (Evolutionary) analysis and alignment of proteins (PALI Database)

5) Name any two databases important in bioinformatics. Mention the type of information which may be obtained from these databases.

Database	Information Available
EMBL(European Molecular Biology Laboratory)	Nucleotide sequence
Nucleotide sequence	Annotated protein sequence
PDB (Protein Database)	Three dimensional structure of proteins
Ribosomal RNAdatabase	rRNAsubunit sequences
PALI database	Phylogenetic analysis and alignment of proteins

5.3.4. SHORT ANSWER QUESTIONS

1) Bioinformatics databases provide resources for gene level sequences such as RefSeq, Homologene, Paralogs and UniGene and BLAST. Which of these would you use as most suitable starting point for:

i) Avoiding redundancy in EST data.
ii) For inferring relations among organisms.
iii) Information retrieved from this resource will be used in designing gene chips.

Ans. i) UniGene database
ii) Homologene database
iii) RefSeq database

2) What kinds of analysis can be undertaken using various bioinformatics tools?

Ans. Processing raw information: The experimentally determined sequence (raw information) is processed using bioinformatics tools into genes, the proteins encoded and their function, the regulatory sequences, and inferring phylogenetic relationships.

Genes: Gene prediction can be done by using computer programs like Gene Mark for bacterial genomes and GENSCAN for eukaryotes.

Proteins: Protein sequences can be inferred from the predicted genes by using simple computer programs.

Regulatory sequences: Regulatory sequences can also be identified and analysed by using bioinformatics tools.

Inferring phylogenetic relationships: Information regarding the relationships between organisms can be obtained by aligning multiple sequences, calculating evolutionary distance and constructing phylogenetic trees.

Making a Discovery: Using the bioinformatics tools and databases, the functions of unknown genes can be predicted.

3) Compare Fluorescence in situ hybridisation technique with Karyotyping for identification of chromosomal translocations.

Ans

FISH	Karyotyping
Interphase chromosomes can be used	Metaphase chromosomes are needed
Easy Technique as it gives colour to the chromosome	No such specific colour

4) Write the steps of BLAST involved in comparison of DNA sequences.

Ans.
- The given sequence is compared with sequences in the database using substitution matrices that specify scores to either 'reward' a match or 'penalize' a mismatch.
- Top scoring matches are ranked according to set criteria that serve to distinguish between a similarity due to ancestral relationship or due to random chance.
- True matches are further examined thoroughly with other details accessible through Entrez and other tools available at NCBI

5.3.5. LONG ANSWER QUESTIONS

1) (a) List three database retrieval tools available from the NCBI. Also mention about the possible use of each.

(b) Which information can be retrieved from the following databases? (i) EMBL (ii) PDB

Ans. (a) Database retrieval tools –

ENTREZ gives access to literature, sequences and structures. **TAXONOMY BROWSER** provides information on taxonomic classification of over 79000 organisms. **LOCUS LINK** carries information on official gene names and other description.

(b) EMBL– nucleotide sequences
PDB - 3D structure of proteins

2) The microarray technique can be used to compare gene products between normal and cancer cells. Explain the principle involved in this technique with a relevant diagram.

Ans. Principle: Microarrays consist of large numbers of DNA molecules spotted in a systematic order on a solid substrate, usually a slide (Fig. 4). The base pairing or hybridization is the underlying principle of DNA microarray. Microarray exploit the preferential binding of complementary single-stranded nucleic acids. A microarray is typically a glass (or some other material) slide, onto which DNA molecules are attached at fixed locations (spots). The type of molecule placed on the array units also varies according to

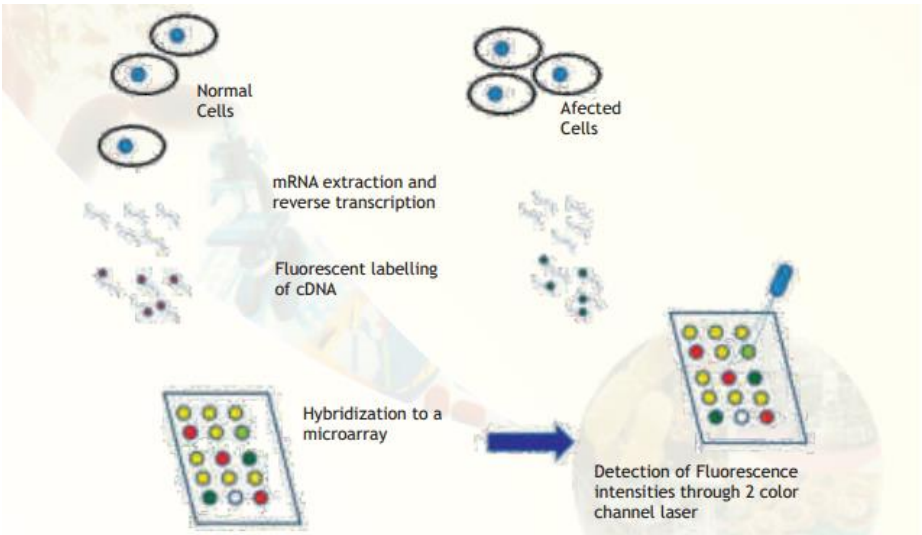


Fig. 4. Major steps involved in comparative microarray hybridization experiments between normal and affected (for example, cancerous) cells are illustrated. Observe that some spots are yellow, meaning that the particular gene is expressed in equal amounts. Some spots are clearly red or green indicating that the particular genes are expressed in only normal or affected condition. Some spots are more greenish or orange meaning that the expression status is not clearly tilted to either side but there is a trend towards either extremities. A few spots may appear blank (no colour).

circumstances. The most commonly used molecule is cDNA, or complementary DNA, which is derived from messenger RNA. Since cDNA are derived from a distinct messenger RNA, each feature represents an expressed gene. In order to detect cDNA, bound to the microarray, they must be labelled with a reporter molecule that identifies their presence. This technique of introducing fluorescent

dyes in DNA and its use in detection of target molecule by hybridization has been previously applied in fluorescence in situ hybridization (FISH).

5.3.6. CASE BASED QUESTIONS

1) BLAST (Basic Local Alignment Search Tool) is a widely used bioinformatics software tool and algorithm for comparing biological sequences. It is primarily used for sequence similarity searches, allowing users to compare a query sequence against a database of known sequences to identify similar sequences and infer functional, evolutionary, or structural relationships.

The BLAST tool employs an algorithm that looks for local regions of similarity between the query sequence and sequences in the database. It calculates alignment scores based on matching residues, gaps, and substitution matrices. BLAST can handle various types of biological sequences, including DNA, RNA, and proteins, and offers different programs tailored to specific sequence types and search requirements.

Answer the questions based on the information given:-

1. Which of the following best describes BLAST?

a) A database management system
b) A sequence alignment algorithm
c) A machine learning algorithm
d) A genome assembly tool

Ans. b) A sequence alignment algorithm

2. What does BLAST stand for?

a) Biological Local Alignment Search Tool
b) Basic Local Alignment Sequence Tool
c) Basic Local Alignment Search Tool
d) Biological Local Alignment Sequence Tool

Ans. c) Basic Local Alignment Search Tool

3. Which type of BLAST is used to compare protein sequences against a protein database?

a) BLASTn
b) BLASTp
c) BLASTx
d) tBLASTn

Ans. b) BLASTp

4. BLAST primarily focuses on:

a) Global alignments
b) Local alignments
c) Multiple sequence alignments
d) Pairwise alignments

Ans. b) Local alignments

5. BLAST can be used to search for similarities between:

a) DNA sequences only
b) Protein sequences only
c) Both DNA and protein sequences
d) RNA sequences only

Ans. c) Both DNA and protein sequences

2) Gene prediction is an important problem for computational biology and there are various algorithms that do gene

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%

prediction using known genes as a training data set. The following table shows Genome size and gene predictions between several organisms-

Based on your observation and analysis, answer the following questions.

(i) Even if we know where the genes are in a given genome, it's difficult to count them due to-

A. splice Variants
B. overlapping genes
C. exons
D. Both A and B

Ans. D. Both "A" and "B"

(ii) Which organism has the maximum part of the genome coding for the proteins?

A. *Escherichia coli*
B. *Saccharomyces cerevisiae*
C. *Caenorhabditis elegans*
D. *Drosophila melanogaster*

Ans. A. *Escherichia coli*

(iii) Part of the genome that encodes for protein in *Homo sapiens* is less than 5 % , one of the probable reason/s for this could be-

A. Repeated Sequence
B. Exons
C. Both "A" and "B"
D. SNP"s

Ans. A. Repeated Sequence

(iv) The relationship between number of chromosomes and genome size in base pairs is:-

A. direct
B. indirect
C. no relationship

D. correlation of 0.5

Ans. C. No relationship

(v) Computational Gene prediction is referred to as:-

A. In –silico Gene prediction
B. In –Vivo Gene prediction
C. In - vitro Gene prediction
D. Microarray prediction

Ans. A. In – silico Gene prediction

(vi) After observing the table, it seems that the relationship between the intuitive complexity of an organism and the number of genes in its genome is:-

A. No simple correlation
B. Simple correlation
C. Inverse correlation
D. Depending on the organism, can be simple or Inverse

Ans. A. No simple correlation

UNIT 6 CELL CULTURE AND GENE MANIPULATION

Chapter 1: MICROBIAL CELL CULTURE AND APPLICATIONS

SUMMARY: IMPORTANT POINTS CONCEPTS & DEFINITIONS

1. A microbial culture works as a factory in converting the raw material into product. This chemical equation for the production of products from reactants in a given period of time is represented as

$$C_wH_xO_yN_z + aO_2 + bH_gO_hN \longrightarrow CCH_aO_bN. + dCO_2 + eH_2O$$

2. For such reactions the microbes need optimum pH, temperature, nutrients (provided by growth medium) to grow and the particular substrate which is converted by the microbe into the desired product.

3. All microorganisms require water, sources of energy, carbon, nitrogen, oxygen and mineral elements.

4. It has been possible to make a nutritive medium using pure compounds in which the microbes can be grown/ cultured under laboratory conditions. Such media are called synthetic media. Culture media is one of the most important factors for culturing cells and tissues. It provides (a) Optimum conditions factors like, pH, osmotic pressure etc.

(b) The chemical constituents which the cells tissues are incapable of synthesizing (unlike microorganisms which can synthesize from simple inorganic substances.)

5. The carbon sources such as carbohydrates, lipids and proteins and Nitrogen sources such as Ammonium salts urea, corn steep liquor or slaughterhouse waste etc in the culture medium from the major sources which provide energy for the growth of microbes.

6. For large scale fermentation processes requiring thousands of litres of culture medium or the substrate, steam is used for sterilization of the fermentation media.

7. Bacteria grow at neutral pH whereas fungi and yeast grow at acidic pH. Before autoclaving the medium, it is important to balance the pH according to the desired need.

8. Fermenters have mechanical stirrers to mix the medium, baffles to increase turbulence, which ensure adequate mixing Forced aeration also provides mixing and the needed oxygen

9. The simple way is to provide a Y-shaped notch or indentation in the sides of the flask. Such flasks are called Baffle flasks. Baffle flasks improve the growth of the microbes by improving the efficiency of oxygen transfer due to increased turbulence of the culture medium for large scale fermentation and microbial transformation the fermenters or bioreactors are used

10. Fermenter is a closed vessel with proper arrangement for aeration agitation, temperature and pH control. They are provided with many controls for the monitoring of physical, chemical and biological parameters that affect the growth by cells.

11. The rapid growth of the microorganisms in the fermenter quickly alters the pH of the medium. Hence the pH of the growing culture should be continuously monitored and maintained by adding acid or alkali as required.

12. Batch culture is a closed culture system containing a limited amount of nutrients. In a fed batch culture, the culture is continuously or sequentially fed with fresh medium without removing the growing culture. Hence over a period of time the volume in the culture vessel goes on increasing. As the name suggests this is the method used in getting a continuous supply of microbial growth and or products.

13. In continuous culture, cells can be grown at a particular growth rate for an extended period of time. At steady state, the cell growth and substrate consumption take place at fixed rate. If the medium is continuously fed to a culture at a suitable rate this steady state is eventually achieved.

14. The Growth rate of the cell remains constant during steady state that means the concentration of cell, metabolite and another nutrient inside the reactor remain constant.

15. Doubling time is the time required for the cell mass or number to double its original value during the balanced growth of the organisms.

Measurement of Cells Mass:

Measurement of cell mass is one of the easiest ways of microbial growth of the organisms.

Measurement of Dry Weight: It is carried out by measuring the dry weight of the cell material in a fixed volume of the culture by removing the cells from the medium and drying them till constant weight is obtained

Use of Spectrophotometer: Cell growth is also measured by measuring the absorbance of cell suspensions in a spectrophotometer. This principle is based on the fact that small molecules scatter light proportionates to their concentration.

Measurement of Cell Numbers: Measurement of growth can also be carried out by counting the cell number. Such counting is usually done with a special microscope slide known as a counting chamber.

16.A microbial culture undergoing balanced growth is like a chemical reaction where substrate is getting converted into a product that is cell mass in this case. The specific growth rate $\mu = 1/X. dX/dt$ is an index of rate of growth of the cells in that particular environment. Specific growth rate can be calculated by plotting dX/dt. vs X and determining the slope of the straight

17 Downstream Processing: After the completion of fermentation the desired metabolites are recovered by separation of cells from the fermentation broth and purification of metabolites with or without cell disruption. Such operation is referred to as downstream processing. The steps involved in isolation of the desired microbial product is

1. Separation of cells from the fermented broth.

2. Cell disruption if the product is intracellular or concentration of the broth if the product is extracellular.

3. Initial purification of the metabolite.

4. Metabolite specific purification where the metabolite of interest is purified to very high degree, and

5. Polishing (bringing to 98 - 100% purity) of the metabolite where it is concentrated and formulated for use.

The Clarified fermentation liquor contains microbial metabolites and extracellular enzymes. They are recovered using the techniques of precipitation, solvent extraction and ion exchange chromatography.

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

18. There are several methods used for preservation of strains as under.

Storage of Agar: Cultures are grown on agar slants or stabs and stored at 5 to 20 °C. These must be sub-cultured at approximately 6-month intervals

Storage in Liquid Nitrogen: The culture is grown and a cryoprotective agent glycerol (10-30%) is added. These are dispensed into sealed ampoules and frozen in liquid nitrogen. (-176^{o}C to -196° C).

Lyophilization: Lyophilization or freeze - drying involves freezing of a culture followed by drying under vacuum. Once the cultures of new strains or commercially/scientifically useful microbes are established they have to be maintained for use for posterity. There are centers which maintain these cultures for posterity, called Culture Collection Centres. The Cultures deposited at these centers are also made available to prospect investigators.

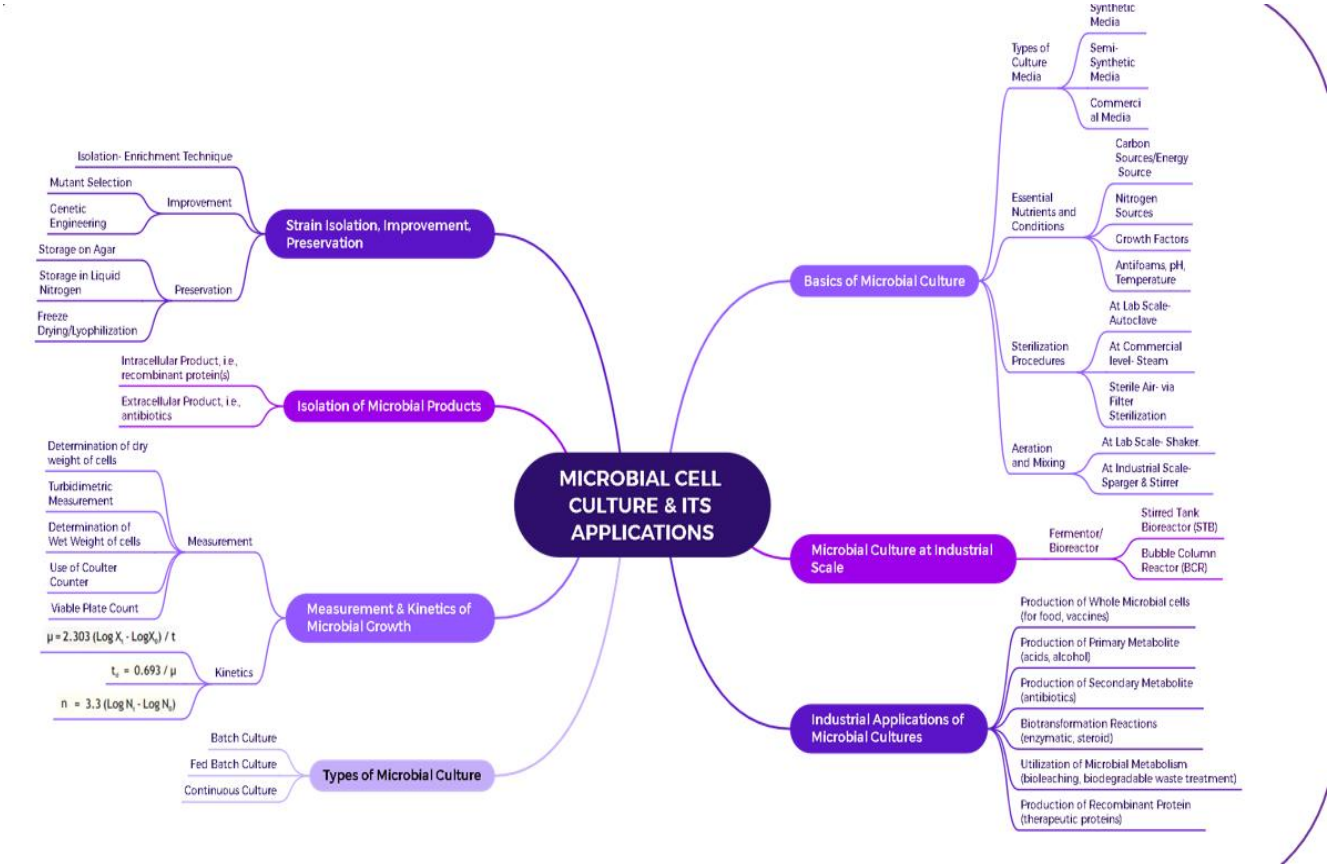
19. Microbial culture has immense potential for the production of very useful compounds. In general, microbial cultures can be exploited primarily in six different ways for the production of metabolites. They are listed below

- 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.

20. 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.

CONCEPT MAPS



MULTIPLE CHOICE QUESTIONS

1. There is no increase in the cell concentration in the lag phase due to the following reasons:

a) Exhaustion of the medium.

b) Space constraint

c) Both “a” and “b”

d) Acclimatization to the new environment.

Ans: d

2. In which phase of growth is the specific growth rate of animal cells calculated?

a) Log phase

b) Lag phase

c) Stationary phase

d) Decline phase.

Ans: a

3. A student adds antibiotics to the animal cell culture medium and still obtains the same growth curve. The probable explanation for it will be:

a) Antibiotics add growth factors and hormones in the medium

b) Antibiotics provide serum for the growth of animal cells.

c) Antibiotics enhance the nutrient content of the medium.

d) Antibiotics don't have any effect on animal cells

Ans: d

4. Which of the following instruments is used for sterilizing the media after it has been prepared?

a) Autoclave

b) Laminar Air Flow Chamber

c) Inoculum Needle

d) Incubator

Ans: a

5. Which phase has the condition of specific growth rate "$\mu = 0$"?

a) Lag phase

b) Log phase

c) Stationary phase

d) Death phase

Ans: d

6. If a culture starts with 50 cells, how many cells will be present after five generations with no cell death?

a.200

b. 400

c. 1600

d. 3200

Ans: c

7. Lyophilization means

a. Sterilization

b. Freeze-drying

c. Burning to ashes

d. Exposure to formation

Ans: b

8. In the medium other than nutrients, if any substance is used in excess, that medium is

a. Enriched medium

b. Special medium

c. Enrichment medium

d. None of these

Ans: a

9. Autoclaving is carried at

a. Dry heat

b. Atmospheric pressure

c. 120 degree Celsius

d. All of these

Ans: c

10. Agar is obtained from

a. Brown algae

b. Red algae

c. Green algae

d. Blue-green algae

Ans: b

11. The condition required for autoclave

a. 120 degree temp.and 15 lbs. pressure for 20 min.

b. 120 degree temp.and 20 lbs. pressure for 30 min

c. 150 degree temp.for 1 hr.

d. 130 degree temp for 2 hr

Ans: a

12. Large vessel containing all the parts and condition necessary for the growth of desired microorganisms is called

a. Bio reactor

b. Auto reactor

c. Impeller

d. None of these

Ans: a

13. The purification and recovery of the production after fermentation is called

a. Upstream process

b. Downstream process

c. Surface fermentation

d. None of these

Ans: b

14. In autoclave, the principle involved is

a. Dry heat

b. Moist heat

c. Steam under pressur

d. Both b and c

Ans : d

15. Antifoam agent is

a. Silicon compounds

b. Corn oil

c. Soyabean oil

d. All of these

Ans: d

16. The apparatus used to maint in a continuous culture

a. Chemostat

b. Autostat

c. Thermostat

d. Both a and c

Ans: d

17. Which is not a method of train preservation?

a) Storage on agar
b) Cryopreservation
c) Storage in alcohol
d) Lyophilization

Ans:c

18. Cultures are grown on agar slants or stabs & stored at 5 to -20 oC. These must be sub-cultured

a. approximately 6-week interval
b. approximately 12-month interval
c. approximately 6-day interval
d. approximately 6-month interval

Ans: d

19. Cultures may be deposited to culture collection centers

1. Because they are not to be stored in laboratory
2. Because they are costly
3. These centers safely maintain cultures for years
4. Can be distributed globally
5. to protect the intellectual property rights of the depositors

Which among these are true

a) Ony 1
b) 1,2,4
c) Only 3,4and 5
d) 1,2,3,4,5

Ans: c

20. Applications of microbial cell culture include

1. Production of whole microbial cells (for food, vaccines).
2. Production of primary metabolites (acids, alcohol).
3. Production of secondary metabolites (antibiotics).

4. Biotransformation reactions (enzymatic, steroid).

5. Exploitation of metabolism (microbial leaching, biodegradable waste treatment).

6. Synthesis of recombinant proteins (therapeutic proteins)

a. Ony 1
b. 1,2,4
c. Only 3,4and 5
d. 1,2,3,4,5,6

Ans : d

21. Single cell protein (SCP) is

1. Application of cell culture
2. A whole cell used as source of protein
3. A selection process
4. An engineered protein

A. Ony 1
B. Only 1,2
C. Only 3,4 and 5
D. 1,2,3,4,5,6

Ans: b

22. Examples of applications of microbes for human use are

1. Expression of humanInsulin in *Escherichia coli*
2. Hepatitis B surface antigen in Yeast

A. Only 1 true
B. both 1,2 true
C. Only 2 false
D. 1 false 2 true

Ans: b

23. The parameter that helps to determine microbial growth is

a. size of culture

b. specific death rate

c. doubling time

d. nutrient media

Ans: c

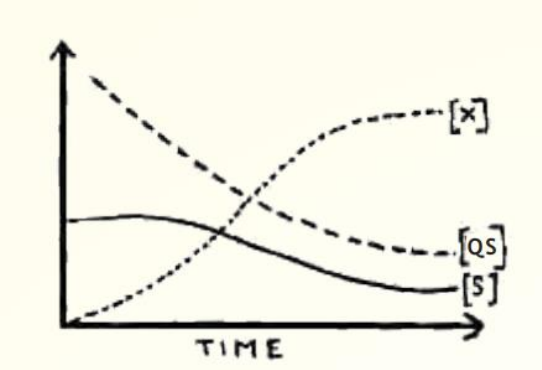
24. Methods used for measuring cell growth

1. measurement of wet weight of cells
2. turbidity measurements,
3. ATP measurement, viable plate count.
4. Coulter counter

A. Ony 1,2
B. Only 1,2,3
C. Only 3and 4
D. 1,2,3,4

Ans: d

25. [X], [S] AND [Q_S] in the diagram are



1. The cell density [X], the concentration of product [S] and cell-specific substrate turnover rate [QS]
2. The cell density [S], the concentration of substrate [X] and cell-specific substrate turnover rate [QS]
3. The cell density [X], the concentration of substrate [S] and cell-specific substrate turnover rate [QS]
4. The cell density [QS], the concentration ofsubstrate [S] and cell-specific substrate turnover rate [QS]

Ans:3

26. The growth medium is designed in such a way that one of the nutrients is in limited quantity. Thus, during the exponential

growth, as this nutrient is exhausted the growth will stop. This is occurring in

a) Batch culture
b) Fed batch culture
c) Both a and b
d) Continuous culture

Ans: d

27. “Growth rate of cells remains constant during steady state operation. This means that at steady state, the concentration of cells, metabolites and other nutrients inside the reactor remain constant. That is, formation of new biomass by the culture is balanced by the loss of the culture from the vessel”. This is occurring in

a) Batch culture
b) Fed batch culture
c) Both a and b
d) Continuous culture

Ans: d

28. There are four general patterns of microbial growth

a. Bacteria grows by chain elongation and branching, yeast divide by budding, fungi divide by binary fission whereas viruses normally do not follow a regular growth pattern
b. Bacteria grows by budding, yeast divide by binary fission, fungi divide by chain elongation and branching whereas viruses normally do not follow a regular growth pattern.
c. Bacteria grows by binary fission, yeast divide by budding, fungi divide by chain elongation and branching whereas viruses normally do not follow a regular growth pattern

d. Bacteria grows by chain elongation and branching, yeast divide by binary fission, fungi divide by budding whereas viruses normally do not follow a regular growth pattern

Ans: c

29. In this formula μ = 2.303 (Log Xt - LogX0) / t, which given below is not correct?

a. μ:The specific growth rate
b. Xt : biomass conc. after time t
c. X0: biomass conc. at the start of the exponential growth
d. t : doubling time (h)

Ans: d

30. *Saccharomyces cerevisiae* has been quite popular because it is safe and scientists have long experience of using this yeast in industrial fermentations but the dis advantage is

a) Detailed information on biochemistry, physiology and genetics of this yeast is also known.
b) Yeast can be manipulated genetically rather easily. P
c) product yields are relatively low at 1-5% of the total protein

1. Only a
2. all of these
3. Only b and c
4. Only c

ASSERTION AND REASON TYPE 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**: Biotechnology produces transgenic microorganisms that act as microfactories for proteins.
   Reason: To produce proteins of human use like insulin. Transgenic microorganisms can be developed.

Ans: a

2. **Assertion**: The desired metabolites can be intracellular or extracellular depending on which to isolate the microbial product cell disruption may be or may not be required. **Reason**: Antibiotics are secreted into the medium while most recombinant proteins accumulate intracellularly.

Ans : b

3. **Assertion:** A fed-batch culture cannot be operated indefinitely
   Reason: The microbes die out eventually due to overcrowding and limited substrate

Ans. A

4. **Assertion**- In most microbiological processes, foaming happens to be a big problem and must be prevented.
   Reason- Excess foaming denatures proteins and provides hindrance to free diffusion of oxygen in the medium.

Ans. A

5. **Assertion**- In a fed-batch culture, over a period of time, overall volume of culture medium in the culture vessel keeps on increasing.
   Reason- This happens because during fed batch culture, fresh medium is continuously added in the culture vessel accompanied

with the removal of an equal volume of the spent medium from the culture vessel.

Ans. c

6. **Assertion**- The expression of a eukaryotic gene inside a prokaryotic host happens to be problematic.
   Reason-For maximizing the production of the foreign protein, the expression vector used must be such that it replicates to high copy number and is stable.

Ans. b

7. **Assertion**- Metagenomics is an approach where the sample containing the microbes e.g. soil/water) is put in a nutritive medium and allowed to grow under suitable environmental conditions in such a way that the growth of microbes of our interest is favoured.
   Reason- Metagenomics is a powerful technique for isolation of desired culturable forms from an environmental sample.

Ans. d

8. **Assertion**- Once a strain producing a novel or desired product has been obtained, it must be appropriately preserved for future use.
   Reason- If not done properly, the strain may be lost through loss of viability or even show decline in the production of the product for which it was isolated.

Ans. a

9. **Assertion**: Industrial fermentations are money making ventrues.
   Reason: They require a thorough research to discover high yields of produce at least possible expenses.

Ans. a

10. **Assertion**: Yeasts should not be used in brewing and baking industries.
    Reason. They produce several harmful products during brewing and baking.

Ans. d

SHORT ANSWER QUESTIONS

1. Write a Chemical equation for the production?

Ans: The Chemical equation for the production of products from reactants in a given period of time is represented as.

2. Name the product whose production becomes commercially viable due to strain improvement through several successive mutations.

Ans: Penicillin.

3. What is the "Baffle flask"?

Ans: The flasks with a V - shaped notch or indentation in the sides of the flasks called "Baffle flask" This improves the growth of the microbes by improving the efficiency of oxygen transfer due to increased turbulence of the agitated culture medium.

4. How are strains cryopreserved?

Ans: They are preserved by storing them in a jar and in liquid nitrogen under -176 to -196 degree Celsius.

5. What is lyophilization?

Ans: Lyophilization or freeze- drying involves freezing of a culture followed by drying under vacuuming results in sublimation of cell water. Lyophilised can remain viable for 10 years or for more.

6. How much is microbial conversion efficiency and why?

Ans: 20-30% as a large part of the substrates are utilized by the organism for growth and multiplication (increase in biomass).

7. Name some Semi Synthetic Media. What do they contain?

Ans: Nutrient broth, trypticase soya broth (T S B) or brain heart infusion (BHI) because They contain complex components such as peptone, beef extract, yeast extract or Casein

8. Name the raw material used as a carbon source in microbial culture.

Ans: Cereal grains, starch, cane molasses, glucose, sucrose, lactose and whey are commonly used as carbon sources.

9. How is the fermentation media sterilized for use in fermenter? Mention the pressure, time and heating temperature.

Ans: Steam is used for sterilization of fermentation media. Steam pressure is held at 1.5 PSI for heating the medium at 120° C for 15 - 20 minutes.

10. What is sparging in a fermenter?

Ans: Sparging involves passing sterile air or steam through the medium

11. Name the energy sources used as nutrients for microbial culture.

Ans: Carbon sources such as carbohydrates, lipids and proteins in the culture medium are the major source which provides energy for growth of microbes.

12. Why are antifoaming agents used in microbial cultures?

Ans: The antifoam agents are added because in most microbiological processes, foaming is produced may be due to the components of the medium or sometimes the microbes themselves produce it. The most common cause of foaming is the presence of proteins in the culture medium. Commonly used antifoams are fatty acids such as olive oil or sunflower oil and silicones.

13. Suggest methods of preserving microbial strains?

Ans: Two methods of preserving microbial strains are:

a. Storage on Agar: Cultures are grown on agar stabs or plates stored at 5 to - 20° C. These must be sub-cultures at approximately 6 month intervals. The time of subculture may be extended to 1 year if cultures are covered with sterile mineral oil.

b. Storage in Liquid Nitrogen: The culture is grown and a cryo-protective agent like glycerol (10-30%) is added. These are dispensed into sealed ampoules & frozen in liquid nitrogen.

c. Lyophilization: It involves freezing of a culture followed by drying under vacuum. This results in sublimation of cell water. It may remain viable for 10 years or more.

14. What is “specific growth rate"?

Ans: The specific growth rate u is depicted by formula $\pi = 1/X\ dx/dt$. It is an index of the rate of growth of the cells in that particular environment. It can be calculated by plotting dx/dt vs X and determining the slope of the straight line.

15. What are the factors which influence the growth of the microorganism?

Ans: Nutritive media of required composition, pH and temperature influence the growth. Besides, aeration and mixing of culture for oxygen transfer and achieving uniform concentration also augment growth rate.

16. How is the process of ultrafiltration used to isolate cells from fermentation broth?

Ans: Where cell settling does not occur, cell removal can be affected by centrifugation. (b) Ultrafiltration. The term ultra-filtration describes processes in which particles significantly greater in size than the solvents are retained when the solution is forced through a membrane of fine pore size, usually less than 0.5 mm.

17. What is a fermenter?

Ans: Fermenter is a vessel, used for large - scale growth of microorganisms under a controlled environment. It is a closed vessel with adequate arrangement for aeration, agitation, temperature & pH control, steaming and drain to remove cultured microbes. The vessel can operate aseptically due to steaming and use of sterile air.

18. Name some Nitrogen sources and trace elements used in nutritive media.

Ans: Nitrogen sources: Ammonium Salts, urea, corn steep liquor or slaughterhouse waste

Trace elements: Cu, Zn, Mn, Mo are also added in nutritive media in small quantities.

19. What Erlenmeyer flask is used?

Ans: It is used in laboratories for culturing microbes.

SHORT ANSWER QUESTIONS -3 MARKS

20. How is continuous culture better than batch or fed batch culture?

Ans: Continuous culture is better than batch or fed batch culture because we can get a continuous supply of microbial growth and/or by - products. In this the growth medium is designed in such a way that one of the nutrients is in limited quantity and during the exponential growth as this nutrient is exhausted the growth will stop. However, just before the nutrient is fully exhausted, a fresh medium containing the limited nutrient is added to it. Hence in continuous culture, cells can be grown at a particular growth rate for an extended period of time. Continuous Culture is most widely used for treatment of liquid waste

21. What is the difference between chemostat and turbidostat?

Ans: In a chemostat, a constant environment is maintained. whereas in a turbidostat a constant cell concentration is maintained.

22. At what PH should bacteria and Yeast & fungi grow in microbial culture?

Ans: Bacteria grows at neutral PH whereas Fungi and Yeast Fungi grow at acidic PH

23. Explain what is meant by Steady State in relation to growth of microbial cultures?

Ans: If medium is fed at a suitable rate to a culture under constant chemical environment (chemostat) and constant cell concentration (Turbidostat) the steady state is achieved. At steady state the cell growth and substrate consumption takes place at fixed rate the growth rate of cells remains constant. The concentration of cells, metabolites and other nutrients inside the vector remains constant. The formation of new biomass by the culture is balanced by the loss of culture from the reactor.

24. Write down the advantages, disadvantages and application of batch culture?

Ans:

The advantages include:

1. Reduced risk of contamination or cell mutation as the growth period is short.

2. Lower capital investment when compared to continuous processes for the same bioreactor volume.

3. More flexibility with varying product/biological systems.

4. Higher raw material conversion levels, resulting from a controlled growth period.

The disadvantages include:

1. Lower Productivity levels due to time for filling, heating, sterilizing, cooling, emptying and cleaning the reactor.

2. Increased focus on instrumentation due to frequent sterilization

3. Greater expense incurred in preparing several subcultures for inoculation.

4. Higher costs for labour and/or process control for this non-stationary process.

5. Larger industrial hygiene risks due to potential contact with pathogenic Microorganisms or toxins,

Common applications for batch cultures include:

1. Products that must be produced with minimal risk of contamination or organism mutation.

2. Operations in which only small amounts of product are produced

3. Processes using one reactor to make various products.

4. Processes in which batch or semi-continuous product separation is adequate.

25. What is the role of water in the culture media?

Ans: Water is the major component of the culture media. For culturing microbes in the laboratory, singe distilled or double distilled water is adequate. For large scale microbial culture in industry, the pH and dissolved salts of the water source has to be considered. Water is also required for the ancillary service such as heating, cooling and rinsing.

26. Why is foaming caused in microbiological processes? How can this be harmful to the process? Name the commonly used antifoams.

Ans: Foaming is caused by metabolites, Proteins and media components. The foaming is harmful as it denatures proteins. Commonly used antifoams are fatty acids such as olive oil or Sunflower oil. Silicones are also used as antifoams

27. How can the desirable characteristics of strain be improved?

Ans: Mutant selection and genetic engineering techniques are used to improve desirable characteristics of strains.

28. How aeration and mixing is done in microbes cultivated in the laboratory?

When microbes are cultivated in the laboratory, aeration and mixing can be easily achieved by putting the flasks on shakers (shake culture) This may be further augmented by the use of baffle flasks. In large scale bioreactors however, transfer of oxygen to microorganisms is particularly difficult because the microorganisms must be well mixed with the medium and the oxygen must be dispersed to achieve uniform concentration. Many fermenter designs have mechanical stirrers to mix the medium, and baffles to increase turbulence, which ensure adequate mixing. Forced aeration also provides mixing and the needed oxygen.

29. What is ultrafiltration?

Ans: Ultra filtration is an alternative to centrifugation. Ultra filtration is the process in which particles bigger in size than the solvent are retained when the solution is forced through a membrane of very fine pore size (<0.5 um). Cells can be concentrated using ultrafiltration.

30. What are excipients?

Ans: The additional ingredients used to stabilize pure metabolites (for marketing or commercial purpose) like sweeteners, dyes, and stabilizing agents are called excipients.

31. What do you mean by coagulation and Flocculation?

Ans: Coagulation: It is the process of formation of small "FLOCS" from dispersed colloids using coagulation agents. Flocculation: It is the process of agglomeration of flocs (formed in coagulation process) into larges settable particles using certain inorganic salts (like CaCl2) or

water soluble organic compounds (Polyacrylamide, Polystyrene, sulfates etc).

32. What do you mean by extracellular and Intracellular in metabolite isolation?

Ans: Extracellular means that metabolites are directly secreted into the medium. Thus their isolation follows an extra cellular recovery path. Example: Streptomycin from *S. Gresius*.

Intracellular means that metabolites are accumulated as intra-cellular. Hence their isolation follows an intra cellular path. Example is Recombinant Insulin from *E. coli.*

33. Name the methods used to recover metabolites and enzymes from clarified fermentation broth.

Ans: Precipitation, solvent extraction, Ion exchange Chromatography etc

34. Define centrifugation in isolation of culture.

Ans: Centrifugation is used to separate particles of 100 - 0.1 um from liquid by gravitational forces. Centrifugation depends upon particle size, density difference between the cells and the broth and broth viscosity. It is used for recovery of Yeast from broth in brewing.

35. Why is forced aeration done during the growth of microorganisms in culture?

Ans: The high concentration of the microbial cells present in the fermenter, can rapidly deplete the soluble oxygen in the medium, creating anaerobic conditions that may not be favorable to the growth of macro-organisms and/ for production of the desired products. So forced aeration is done

36. What are the functions of microbial culture collection?

Ans: Cultures are deposited at culture collection centers. These centers maintain cultures for posterity made available to prospective investigators. The culture collection centers are governed by stringent rules and regulations to protect the intellectual property rights of the depositors. Some of the well-known culture collection centers are:

i. ATCC (Americal Type Culture Collection center)

id. NCIB (National Collection of Industrial Bacteria, British).

ili. DSM (Deutsche Sammlungvon Mikroorganismen and zelkulturen, German)

iv. MTCC (Microbial type culture collection and Gene bank - located at Institute of microbial Technology, Chandigarh.

37. What is the name and location of the National Culture Collection Centre of India?

Ans: MTCC located at Institute of microbial Technology, Chandigarh.

38. While culturing microbes in the laboratory in a 500-ml conical flask, what measures do you suggest to enhance their growth?

Ans: Growth of microbes can be improved by Improving the design of the flasks. One of the simplest ways is to produce a V - shaped or indentation in the sides of the flask which improves the growth of the microbes by improving the efficiency of oxygen transfer due to increased turbulence of the agitated culture medium by the use of shakers - the continuous agitation of the culture medium improves greatly the efficiency of the oxygen transfer and improves the growth of the microbes.

41. What is the role of shakers in microbial culture?

Ans: The shaker provides the continuous agitation of the culture medium. The continuous agitation improves the growth of microbes due to efficient oxygen transfer.

42. Why does the PH of the medium need to be controlled during the microbial culture?

Ans: The growth rate of organisms is influenced by the PH of the medium. The PH is therefore balanced before autoclaving the medium.

In fermenters, PH is altered quickly due to rapid growth of the microorganism. Therefore, the growing culture is continuously monitored and maintained as per requirement by adding acid or alkali.

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

Ans:In microbial culture a steady state is achieved when the cell growth and substrate consumption take place at a fixed rate. Growth rate of cells remains constant during steady state. The concentration of cell metabolites and other nutrients inside the reactor remains constant at steady state. The new biomass produced in culture is balanced by loss of the culture from the vessel.

45. What is time doubling time?

Ans; It is the time required for the cell mass or cell number to double its original valve during the balanced growth of the organism.

LONG ANSWER QUESTIONS -5 MARKS

46. Name and describe the equipment used for microbial cell culture for laboratory level and for large scale fermentation.

Ans:

Laboratory level fermentation: Baffle flask, Shakers

Description:

Laboratory level fermentation: In laboratories, microorganisms can be cultured in test on a very small scale. Erlenmeyer flasks are used for cultures with volumes from 100 to 1000 ml. These flasks are resistant to breakage due to heating or chemical attack and hence can be easily used for fermentation. Laboratory-scale fermentation processes can be improved by better design of flasks and use of shakers, discussed as under –

a. Baffle Flask: One of the ways of improving the design of flasks is the incorporation of baffles. In a glass flask, you can have the base and wall indented (softened by heat) with a narrow edge at 3-4 places to increase turbulence when the flask is used to grow Microorganisms on a shaker. These types of flasks are called baffle flasks. Baffles improve the growth of the microbes by improving the efficiency of oxygen transfer due to increase in turbulence in the culture medium. Larger flasks and even industry-scale fermenters also have baffles.

b. Shakers: Continuous agitation of the culture medium is another way of improving the efficiency of the oxygen transfer. This greatly increases culture growth. Shakers are used for this purpose. Growth of the microbes by improving the efficiency of oxygen transfer due to increase

in turbulence in the culture medium. Larger flasks and even industry-scale fermenters also have baffles.

b. Shakers: Continuous agitation of the culture medium is another way of improving the efficiency of the oxygen transfer. This greatly increases culture growth. Shakers are used for this purpose Large Scale fermentation:

a. Fermenter: Fermenters (bio reactors) are used for industry level fermentation. Fermenters are closed vessels with arrangements for aeration, agitation, control of pH and temperature including inflow and outflow vents. Bioreactors are capable of handling thousands of litres of culture media and can operate aseptically for a number of days. They also need a set-up time (as the same vessel can be used for different types of cultures) whenever a new batch is to be run. Diagram: Refer figure 1 (basic design of a fermenter, page no 104, of microbial cell culture in the textbook

b. Stirred tank bioreactor: Is the most common type of bioreactors. The culture medium is stirred with an impeller. A high concentration of the microbial cells in the bioreactor can rapidly deplete the soluble oxygen in the medium. This can create anaerobic conditions and hence the process can suffer. Forced aeration is resorted to for improved mixing and thus oxygen transfer.

c. Airlift bioreactor: Is another type of bioreactors. In such bioreactors, the motion of injected gas through a central tube (draft tube) causes circulation with fluid re-circulating through the annulus between the tube and the tower or vice versa. Airlift bioreactor works without an impeller and with forced air-flow at high pressure. Vertical flow of air, which continually flows inside the chamber, is created. Although oxygen transfer happens in stirred tank also, the oxygen transfer rate is much higher in airlift bioreactors.

47. What is the use of coulter counter?

Ans: Coulter counter is an electronic instrument for direct counting of microbial cells in suspension.

48. What do you understand by strain improvement?

Ans: The process of strain isolation only identifies a strain capable of producing desired product. But it does ensure that it produces a strain in sufficient quantity to be economically viable. The techniques of

classical genetics and genetic engineering are used to improve the desirable characteristics of the strain, as under.

a. Mutant selection: In this method, the strain is exposed to chemicals like Nitrosoguanidine (N T G) or physical agents like UV rays. These act as mutagens and then suitable mutants are selected. This process is a hit and trial and several successive steps may be needed to achieve the desired mutation. Example: The production of strain of *Penicillium chrysogenum* was achieved through several successive mutations. This strain was capable of producing nearly 100 times the concentration of Penicillin produced by the original strain. This made the production of Penicillin commercially viable. b. Genetic Engineering Technique: It is possible to produce bacterial strains through genetic engineering that can be used to produce plant and animal gene products.

49. What do you mean by downstream Processing? Write down the steps involved in this Process.

Ans: Once the fermentation is complete, it is necessary to recover the desired metabolite. The recovery of desired metabolites involving the separation of cells from the fermentation broth and purification with or without disruption of cells is called downstream processing. The steps involved in isolation of the desired microbial product are

1. Separation of cells from fermentation broth.

2. Cell disruption if the product is intra cellular or concentration of the broth if the product is extracellular.

3. Initial purification of the metabolite.

4. Metabolite specific purification where the metabolite of interest is purified to very high degree polishing of the metabolite where it is concentrated and formulated for use. (Bringing 98-100% purity)

50. What is the difference between Batch culture and fed Batch culture?

Ans: a. Batch culture It is a closed culture system containing a limited amount of nutrients. After bacterial inoculation the organism shows normal growth phases ie, Lag, Log, stationary and decline Phases. Growth results in consumption of nutrients and excretion of microbial products. At stationary phases, the growth declines to zero. The growth cells are continuously exposed to a changing environment.

Example: Culturing microbes in a laboratory in an ordinary flask.

b. Fed- Batch Culture: If a batch described above is continuously or sequentially fed with fresh medium without removing the growth, the culture is called fed-batch culture. Over a period of time the volume in the culture vessel goes on increasing. Fed-Batch culture is preferred when high substrate concentration causes growth inhibition. To overcome this problem, substrate is fed at concentrations below its toxic level to achieve growth. By using fed-batch culture, high cell densities can be achieved in the same reactor volume comparison to the normal batch culture. Ex. fed batch culture is an ideal process for maximum production of intracellular metabolites from the same volume of the reactor.

51. What are the main aspects of consideration for safety specific to Biotechnology?

Ans: The important considerations with regard to safety are Pathogenicity: refers to the ability of an organism to cause disease to the host. Ability means the genetic component of the Pathogen. Possibility of toxicity and allergies associated with microbial production.

- Problem associated with the disposal of spent microbial biomass.
- Safety aspects associated with contamination, infection or mutation of process strains.
- There are fears that genetically engineered microorganisms could escape from the laboratory to the outside world and create unpredictable and perhaps catastrophic consequences.
- Then such release may upset the balance of nature or foreign DNA, in new microorganisms could alter its metabolic activity in undesirable and unpredictable ways.
- Guidelines have been established to ensure safe working practices and levels of containment based on potential hazards.

52. Write down the various applications of microbial culture technology.

Ans:

1. Production of whole microbial cells (food, vaccines)

2. Production of primary metabolites (acids, alcohol)

3. Production of secondary metabolite (antibiotics)

4. Biotransformation reactions (enzymatic, steroids)

5. Exploitation of metabolism (microbial leaching and waste treatment)

6. Recombinant proteins (Therapeutic proteins, gene delivery vectors/DNA) Expression of human insulin in E. Coli and hepatitis B Vaccine in Yeast are the most notable examples of application of microbes for human use.

53. Explain what is meant by GRAS and GMP?

Ans:a. GRAS: Food and Drug Administration (FDA) in the USA has restricted certain microorganisms for their use as a source of protein or for introducing genes. These microorganisms are designated as generally regarded as safe. The GRAS listed microbes are non-pathogenic, non toxic and generally should not produce antibiotics. An organism or a product with GRAS status is exempted from the statutory market approval requirements.

An organism is included in the GRAS list based on its history of safe use or after an appropriate safety assessment. Many of the GRAS organisms found in nature have been genetically modified with beneficial genes to improve the quality of soil as well as to improve the quality of the environment. EPA (environment protection agencies) monitors on regular basis the ecology around the area where these organism have been released

Example:

Lactic acid bacteria (LAB), daily ingested by humans are widely used in the food Industry for the manufacture of fermented products *Saccharomyces cerevicae* is extensively used for any recombinant proteins

b. GMP: The process design in manufacturing should also include factors that make it easier to implement good manufacturing practices. The bioreactor design based on GMP should ensure Adequate mixing of microbial culture to ensure sufficient supply of nutrients and prevent accumulation of toxic metabolites. To avoid excessive agitation this may cause mechanical damage to the roller.

CASE BASED QUESTIONS

Read the following passage and answer **any 4** of the following 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

Ans: 3

2) Choose the correct sequence of the procedure of Metgenomics 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 B

Ans: 4

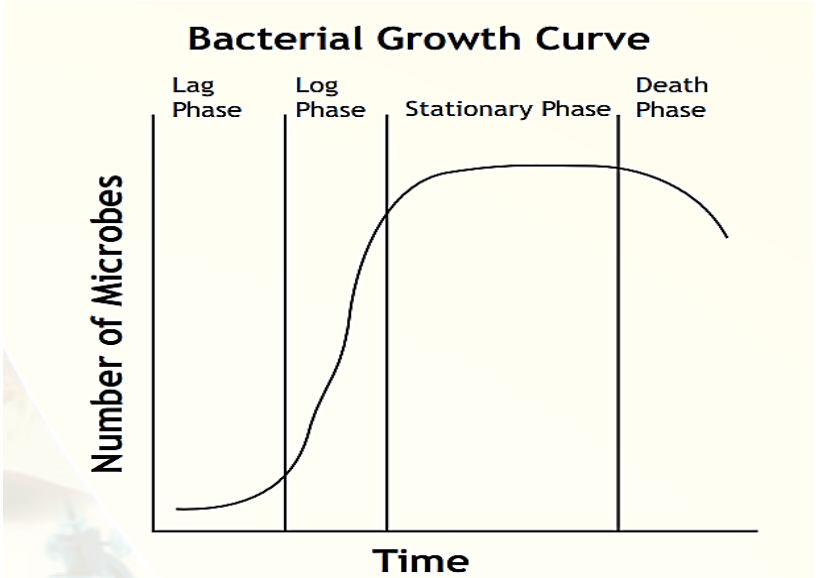
3) What is the primary focus of metagenomics

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

Ans: b

2. Microbial growth kinetics

2. 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

Ans:2

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

Ans: A

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

Ans: d

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

2. Coulter Counter
3. Haemostat
4. Petroff-Hausser chamber

Ans: 1

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 results

Defined (Synthetic) Media

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 (Undefined) Media

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

Ans: b

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

a) Beef extract

b) Peptone

c) Agar

d) Yeast extract

Ans .C

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

a. Simple

b. Complex

c. Defined

d. Natural

Ans: b

5. 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

Ans: 4

4. Fermenter

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

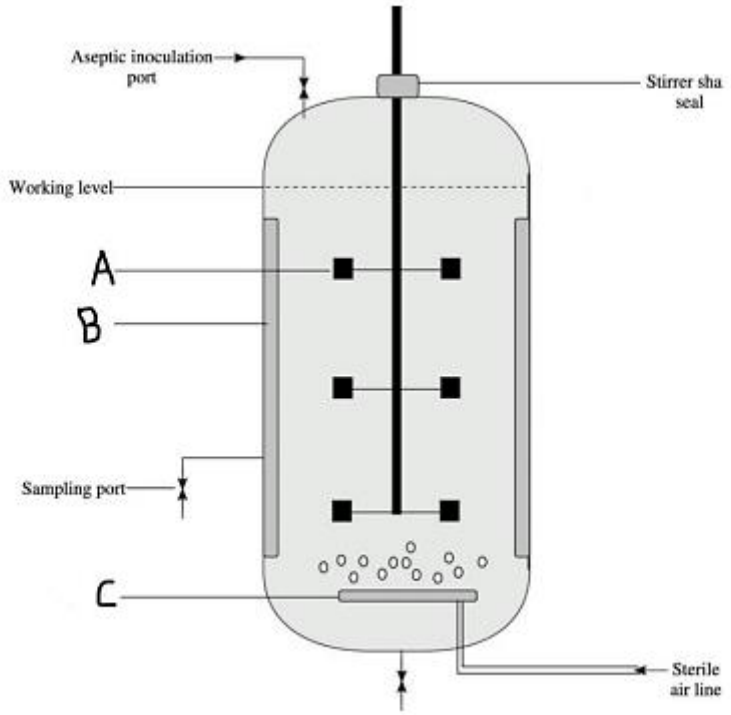
The bioreactor is the heart of any biochemical process as it provides an environment for microorganisms to obtain optimal growth and produce metabolites for the biotransformation and bioconversion of substrates into desirable products.

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 labeled 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

Ans: d

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

a) Open

b) Closed

c) Isolated

d) Semi-closed

Ans: b

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

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

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

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

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

Ans: b

5. Strain improvement

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 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 results in improved characters.

3. “When a eukaryotic gene is introduced into a host bacterium, it may not be always 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 5: 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 tip.

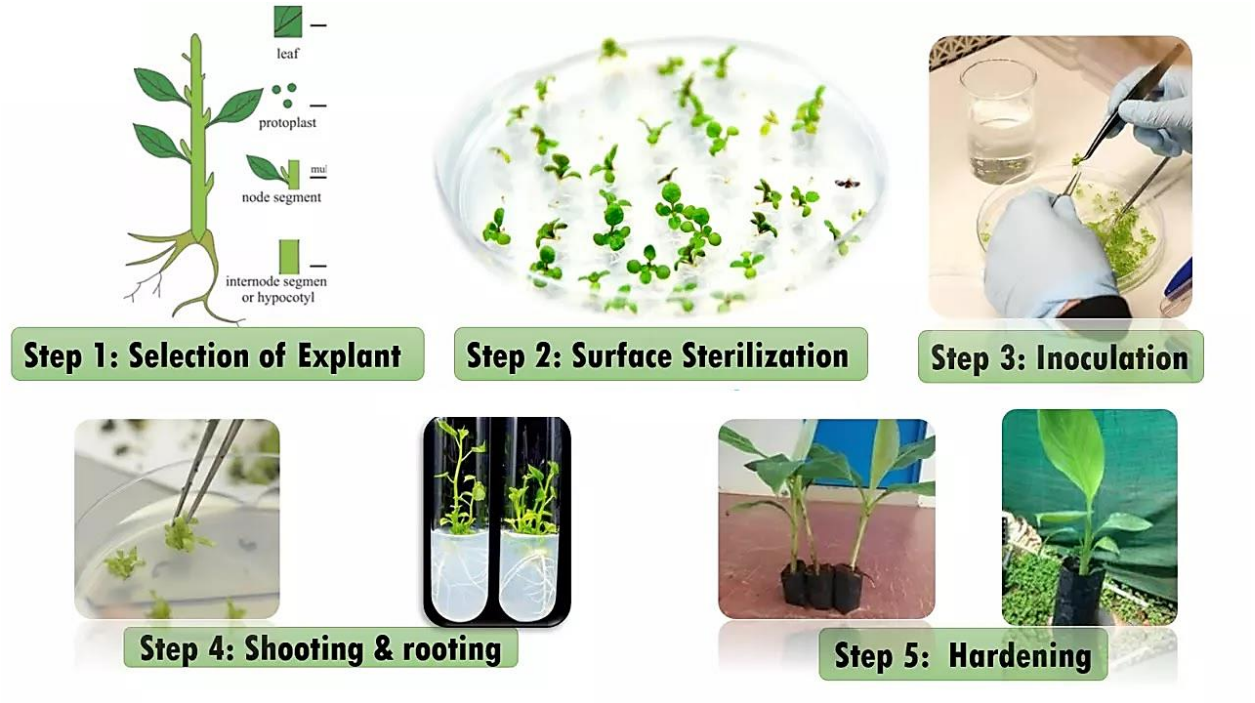
b) Surface sterilization of the explants by disinfectants (example sodium hypochlorite) and then washing the explants with sterile distilled water.

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 1962.9. 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 call.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.

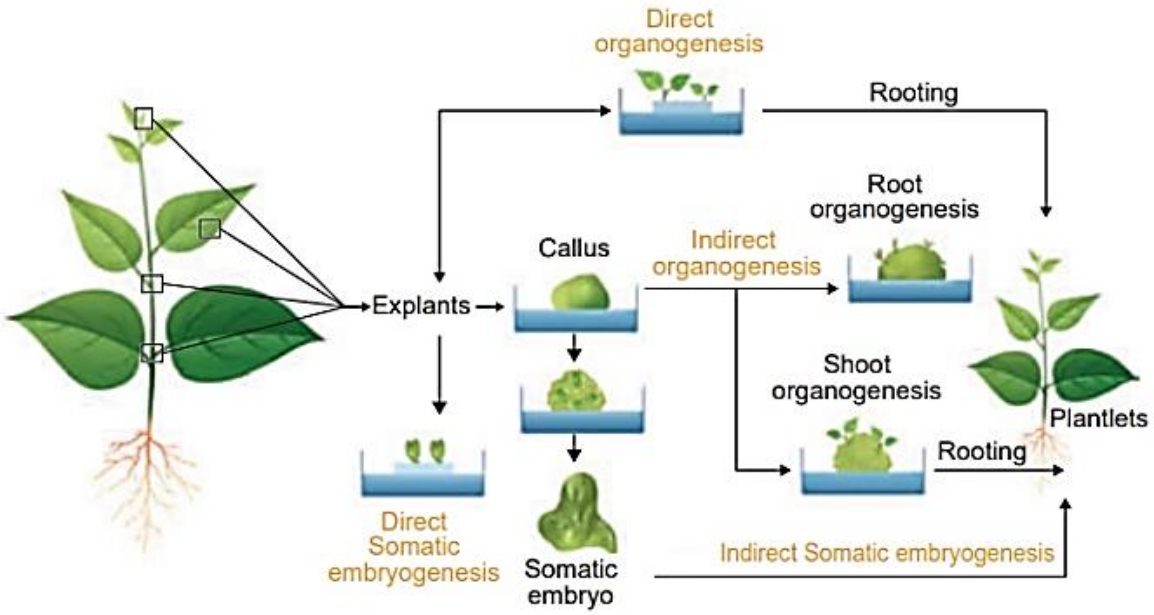


Figure 5.10: Plant Regeneration Pathway

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 inter genetic 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, dimethylsulfoxide, 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,ex: 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.

40. In order to solve the food related problems and to increase the food production and crop productivity, GM or genetically modified crops are becoming very popular.

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.

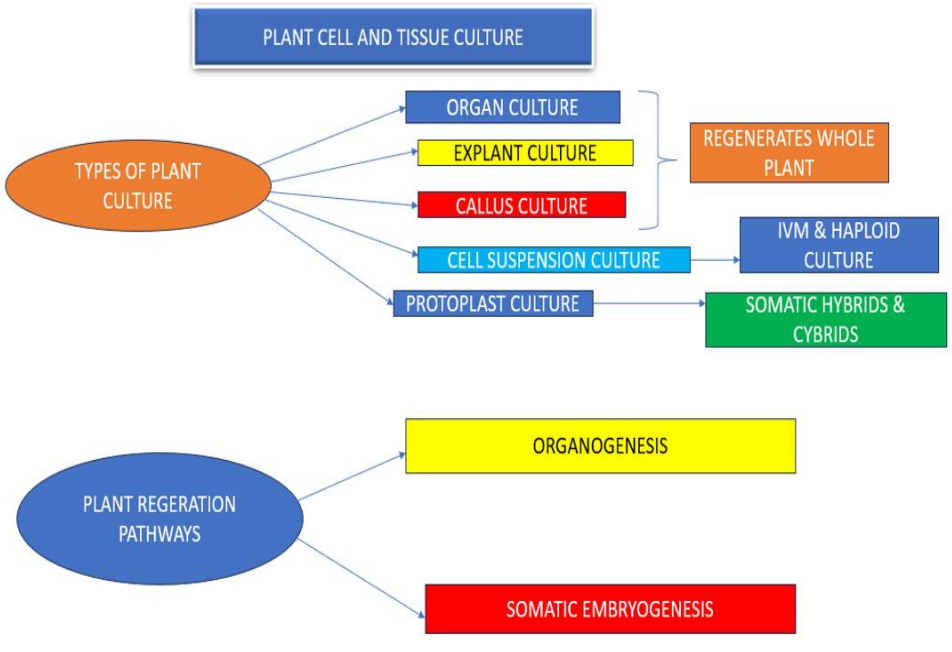
5. The GM crops may change the fundamental vegetable nature of plants as the genes from animals (e.g. fish or mouse) are being introduced into crop plants.

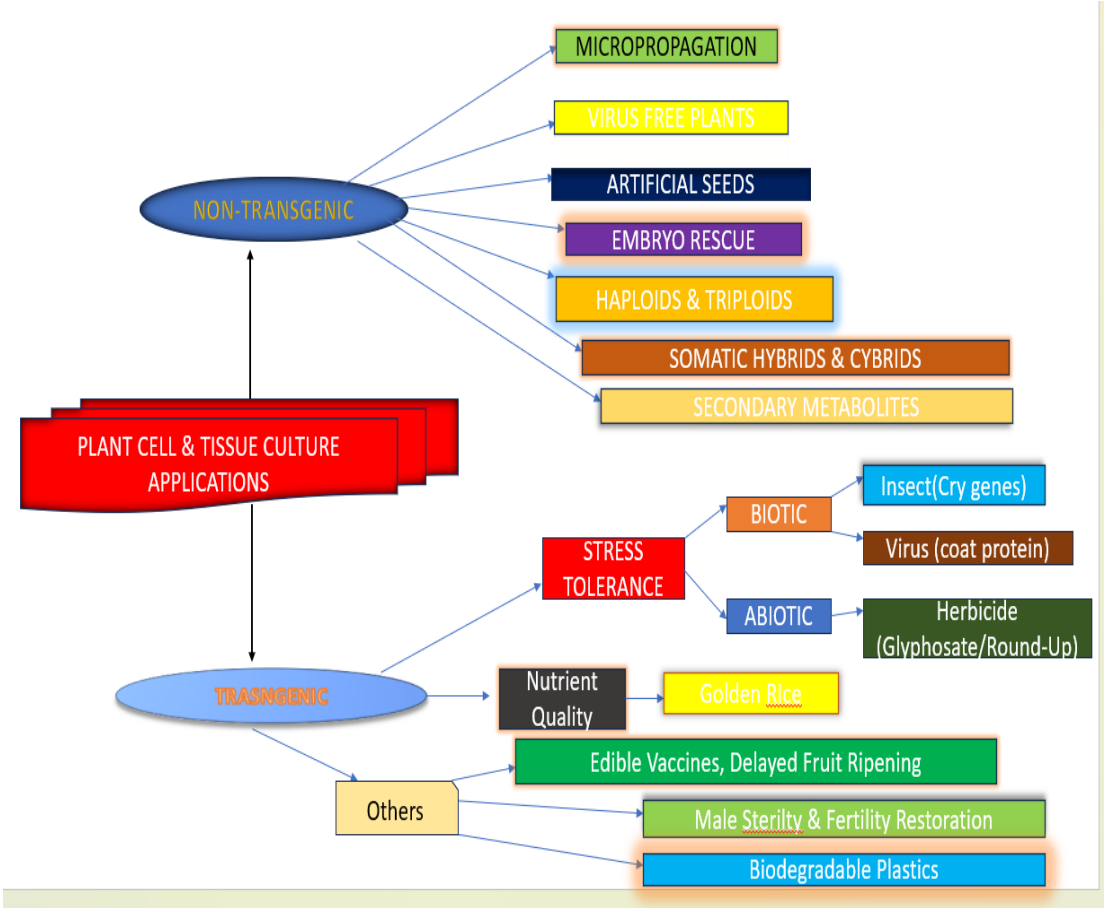
6. The antibiotic resistance marker genes used to produce transgenic crops may horizontally transfer into microbes and thus exacerbate the problem of antibiotic resistance in human and animal pathogens.

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.

CONCEPT MAPS





MULTIPLE CHOICE QUESTIONS

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

(a) Bonner

(b) Laibach

(c) Haberlandt

(d) Gautheret

Ans: (c) Haberlandt.

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

(a) Meristem

(b) Protoplast

(c) Axillary buds

(d) Cell suspension

Ans:(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

Ans: (b) Auxin and cytokinin.

4. What is Dimethyl sulfoxide used for?

(a) A gelling agent

(b) Cryoprotectant

(c) Chelating agent

(d) An Alkylating agent

Ans: (b) Cryoprotectant.

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

Ans: (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

Ans: (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

Ans: (b) Development of an organ from a cell in a culture medium.

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

Ans: (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

Ans: (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.

Ans: (a) Plants raised in tissue culture.

11. Haploid plants can be obtained from_____.

(a) Anther culture

(b) Bud culture

(c) Leaf culture

(d) Root culture

Ans: (a) Anther 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

Ans: (b) Hormones.

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

(a) Embryo

(b) Nucleus

(c) Apical bud

(d) Entire anther

Ans: (d) Entire anther.

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

(a) Agrobacterium

(b) Plasmid

(c) Cosmid

(d) Phasmid

Ans: (a) Agrobacterium.

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

(a) Ethylene

(b) Cytokinin

(c) Auxin

(d) Gibberellin

Ans: (c) Auxin.

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

Ans: (a) Nucleus of one species but cytoplasm from both the parent species.

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

Ans: (c) Synthetic media.

18. Which of the following chemicals are most widely used for protoplast fusion?

(a) Mannitol

(b) Polyethylene glycol

(c) Sorbitol

(d) Mannol

Ans: (b) Polyethylene glycol.

19. Which of the following plant cells shows totipotency?

(a) Cork cells

(b) Meristem

(c) Sieve tube

(d) Xylem vessels

Ans: (b) Meristem.

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

Ans: (b) An unorganized actively dividing mass of cells maintained in culture.

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

Ans: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

Ans:b) Plant cell – cell wall

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.

Ans : a

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

Ans : 4

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
b) Organelle uptake\
c) Both a and b
d) Only b

Ans : c

26. I. Preservation of the genetic resources by Seeds, vegetative prop gules, 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

Ans : 4

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
2. both a and b
3. only b
4. another strategy is to be used

Ans : b

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

Ans : 4

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) None of the above

Ans: a

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

Ans : d

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 toti-potency?

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.

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. These are-Steps

i. Initiation of culture.

ii. Shoot formation.

ii. 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: -

Economic

Conserve nature

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 & intergenetic 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 inter-cellular 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, Ex-crygene.

23. What was the name of the first gene available for genetic engineering of crop plants for pest resistance?

Ans: CryIAC gene

24. The Agrobacterium is considered as 'Natural genetic Engineer of plants' Comment.

Ans: Agrobacterium is known as the natural genetic engineer of plants, since these bacteria have natural ability to transfer T-DNA of their plasmids into plant genome upon injection of cells at the wound site and cause an unorganized growth of a cell mass known as crown gall.

F1 plasmids can be used as gene vectors for delivering useful foreign genes into target plant cells and tissues. The foreign gene is cloned in the T-DNA region of Ti-plasmid in place of unwanted sequences.

25. 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 lipstics 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 Example.

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.

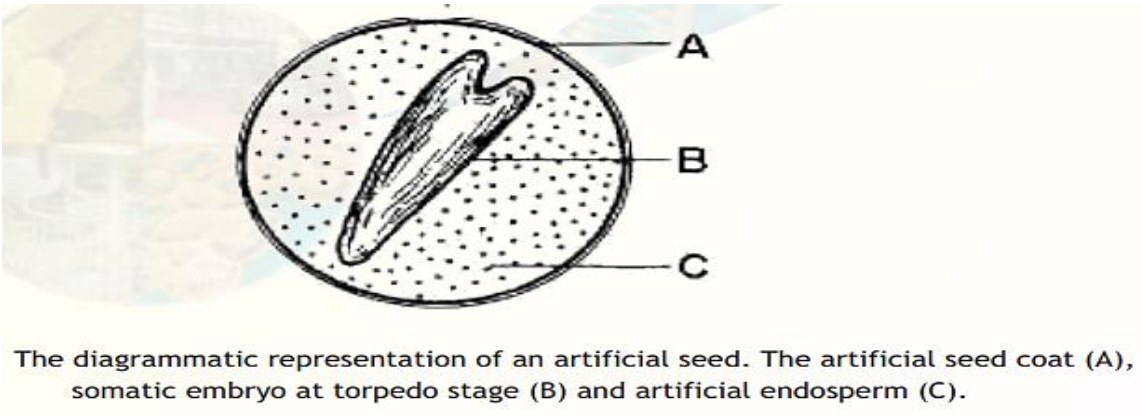
33. How do the transgenic plants used as factories to produce Biodegradable plastics?

Ans: Transgenic plants are used as factories because: Genetically engineered *Arabidopisis* plants produce PHB (polyhydroxybutyrate) 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 fiber 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. Tr 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.

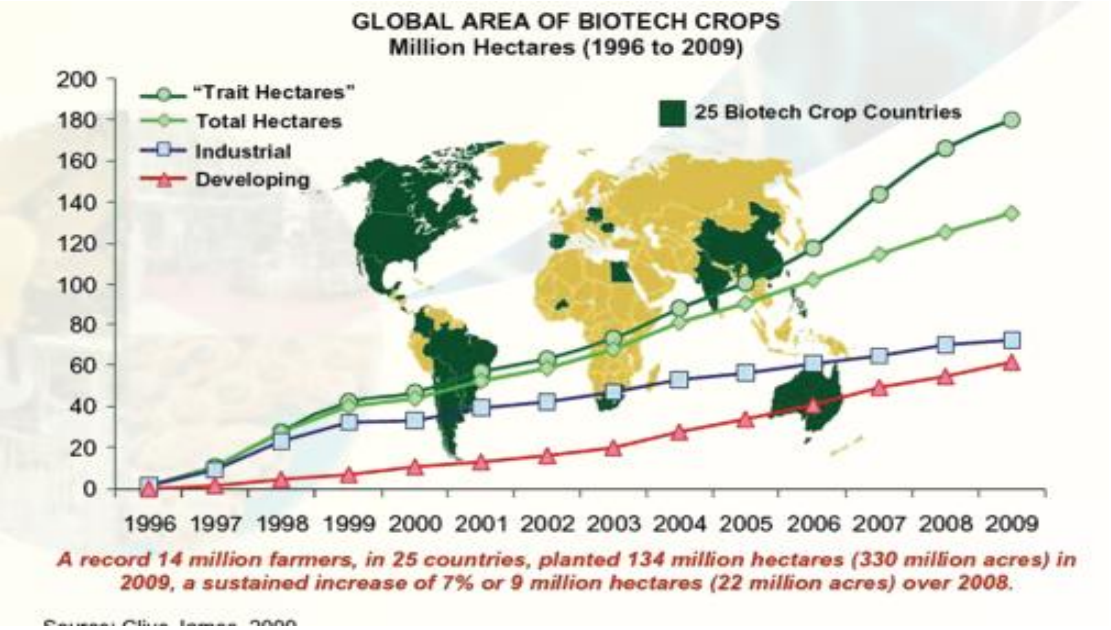
7. The antibiotic resistance marker genes used to produce transgenic crops may horizontally transfer into microbes and thus exacerbate the problem of antibiotic resistance in human and animal pathogens.

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

Ans: b

2. Somatic embryoids 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

Ans. b

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

Ans. D

4.The encapsulation of somatic embryos can be carried out by

A.automatic encapsulation process

B.gel complexation

C.both (a) and (b)

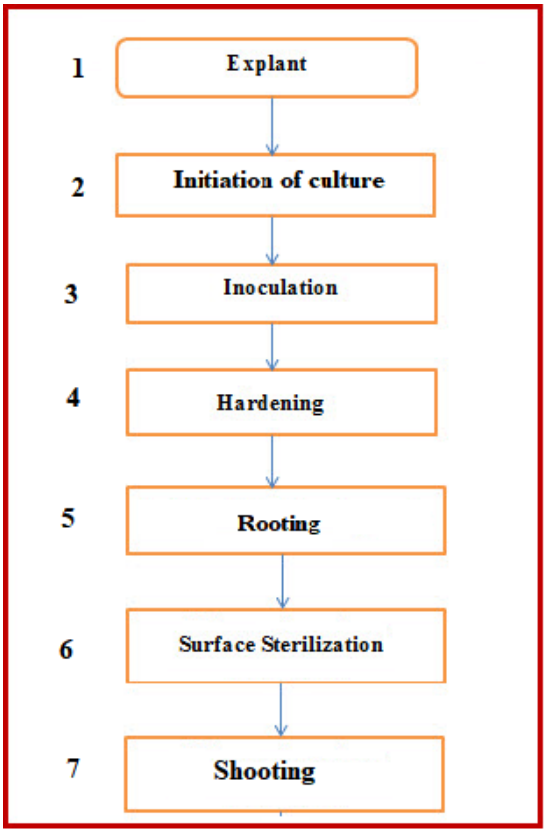
D.coating proteins

Ans. C

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 labour 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 cuttings, budding, grafting, corms, tubers and other vegetative propagules. The main problem with this method is that it is labour-intensive, low productivity and seasonal. Thus, tissue culture method of plant propagation, known as 'micropropagation' 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.

Chapter 6: ANIMAL CELL CULTURE AND APPLICATIONS

SUMMARY: IMPORTANT POINTS, CONCEPTS & DEFINITIONS

1) The process of growing of cells under laboratory conditions is called **Cell Culture**. It is carried out in vitro ('within glass') as opposed to in vivo ('within the living').
2) A **homogenous** population of cells derived from a single parental cell is called a clone. Therefore, all cells within a clonal population are genetically identical
3) The **growth rate of animal cells** is relatively slow and usually require 18 to 24 hour to divide. This makes the animal cell culture vulnerable to contamination, as a small number of bacteria would soon outgrow a larger population of animal cells
4) The animal cell culture became a routine laboratory technique in 1950s after George Gay established the first human cell line **(HeLa**) from cervix cancer
5) **Advantage**s of animal cell culture:
    - Homogenous genetic population.
    - Controlled physico-chemical environment.
    - Easy to add genes (Transfection) or regulate protein levels (RNAi).
    - Available in adequate numbers to do chemical study.
    - Easy production of biopharmaceuticals.
    - No ethical clearance required.
    - Cost effective screening assays.
6) **Disadvantages** of animal cell culture
    - Small size (high sensitive techniques to detect changes).
    - Scale-up is challenging.
    - May not represent in vivo phenotype/genotype.
7) Large proteins or glycoproteins of therapeutic value could not be produced in bacteria. This prompted the **usage of animal cell lines for large-scale production of therapeutic proteins.**
8) **Feature**s of animal cell culture:
    - Animal cells can be grown in glass or plastic vessels.
    - Nutritive media that need to be is periodically replenished.
    - Depending on the tissue they have been isolated from, they can be grown only for limited generations even in the best nutritive media.

- Another important feature of animal cells is that they divide and fill the surface of the culture vessel and then stop growing (**contact inhibition)**
- In animal cell the major difference is the absence of cell to cell interactions, cell-matrix interaction, lack of three dimensional architecture and alteration in hormonal and nutritional environment

9) Differences in growth patterns in normal versus cancer cells are utilized by Oncologists (cancer biologists) to determine whether tumours are cancerous or not using **`Colony formation assay'**

10) The cell culture can be classified as primary cell culture and secondary cell culture.

11) The maintenance of growth of the cells under laboratory conditions in suitable culture medium is known as **primary cell culture**

12) Primary cells can grow either as an adherent **monolayer** or in a suspension

13) **Adherent cells** are usually derived from tissues of organs such as kidney where they are not mobile and are embedded in connective tissue

14) **Suspensio**n cells do not attach to the surface of the culture vessel. Virtually all suspension cultures are derived from cells of the blood system

15) **Primary cells obtained** by disaggregating the tissues mechanically or enzymatically to produce suspension cells.

16) The **most frequently used enzymes** for separating cells from a given tissue (dispersion) are crude preparations of **trypsin and collagenase** that cleave the proteinaceous cementing material between cells in a tissue

17) The **drawbacks of primary culture** are that they are time consuming and require the use of live animals or fresh tissue. There can be considerable variation from one preparation to another particularly if prepared by different people. T

18) Once the primary culture is sub cultured then it becomes **secondary culture** or secondary cell line.

19) **Sub culturing** or "splitting cells," is required to periodically provide fresh nutrients and growing space for continuously growing cell lines

20) The **frequency of subculture** or density of cells to be plated, depends on the characteristics of each cell type. If cells are split too frequently or at too low a density, the line may be lost. If cells are

not split frequently enough, the cells may exhaust the medium and die

21) **Sub-culturing involves**: removing the growth media, washing the plate, disassociating the adhered cells, usually enzymatically (e.g. with trypsin), although some cells may be removed by repeated pipetting or gentle scraping), and diluting the cell suspension into fresh media.

22) **Rous and Jones** were first to introduce proteolytic enzyme trypsin for the subculture of adherent cell

23) Sometimes, certain cells of these secondary cell cultures can spontaneously become altered **(transformed**) and give rise to **continuous cell lines** which show immortality

24) Cell lines are categorized into two types **as finite cell line and continuous cell line**. Finite cell lines are those cell lines which have a limited life span and grow through a limited number of cell generations.

FINITE CELL LINES	CONTINUOUS CELL LINES
Show Mortality	Immortal
Have Contact Inhibition	No contact Inhibition
Show Anchorage Dependence	No Anchorage dependence
Form Monolayer	Multilayer formation
Slow growth rate (24 to 96 Hours)	12-14 hours for cell division
Low Density	High Density (Smaller and rounder)
Ex: Normal cells	Ex: **Hela, CHO, Cos-1** (Mammalian Cell lines)

25) Cell lines transformed under in vitro conditions give rise to **continuous cell lines.**

26) Most mammalian cell cultures are grown in **incubators maintained at 37°C**

27) The **pH is important** to maintain proper ion balance, optimal functioning of cellular enzymes and binding of hormones and growth factors to cell surface receptors.

28) The **regulation of pH** is done using a variety of buffering systems.

29) Salt, glucose and amino acids in the growth media determine the **osmolality of the medium**.

30) **Culture medium** is one of the most important factor for culturing cells and tissues. The most commonly varied factor in culture systems is the growth medium

31) **Nutrient media** contains inorganic salts but serum is essential for animal cell culture and contains growth factors which promote cell proliferation.

32) **sodium bicarbonate** in the medium perform **dual roles**. It is used as a carbonate source and it play an important role in maintaining the appropriate pH and osmolality.

33) **pH indicator** is very helpful in monitoring the pH of the culture medium in an incubator.

34) **Phenol red** (pH indicator) into yellow in Highly acidic conditions while highly alkaline conditions turn the phenol red into pink color.

35) **Serum** is one of the most important components of animal cell culture, as it supports cell proliferation and their attachment to culture vessels. Serum is also a source of various amino acids, hormones, lipids, vitamins, polyamines and salts containing ions such as calcium, chloride, ferrous, ferric, potassium etc.

36) **Antibiotics such as penicillin and streptomycin** are often used in culture medium to control the growth of bacterial and fungal contaminant

37) A cell culture laboratory should have **equipments** like tissue culture hood, CO incubator, inverted microscope, Centrifuge etc. for doing animal cell culture work

38) The **Laminar Air Flow (LAF) hoods** allow the work area to be free of such contamination.

39) A **LAF hood** essentially performs **two functions:**

1. Protects the tissue culture from the operator (by providing a sterile environment).

2. Protects the operator from the tissue culture (from possible infection risk)

40) LAF hood: On the basis of the nature of cells tissue culture hoods are grouped into three types as I, II & III

41) The LAF hoods have continuous displacement of air that passes through a high efficiency particle air **(HEPA) filter** that removes particulates from the air

42) **CO2 incubator** maintains the constant temperature, maintain the sterility of the chamber, an atmosphere with a fixed level of carbon dioxide and high relative humidity

43) **A pan of water** is kept at all times **in the incubator chamber** to maintain high relative humidity and prevent desiccation of the culture medium and maintain the correct osmolality

44) The animal cells are grown in an atmosphere of **5-10% CO2**

45) An **inverted microscope** is used for visualizing cell culture in situ.

46) Several medically important protein pharmaceuticals have been produced using animal cell culture and recombinant DNA technology

47) **t-PA**- Tissue plasminogen activator was the first drug to be produced by mammalian cell culture, which is safe and effective for dissolving blood clots tPA is a serine protease that catalyzes the conversion of plasminogen to plasmin which is responsible for dissolving blood clots.

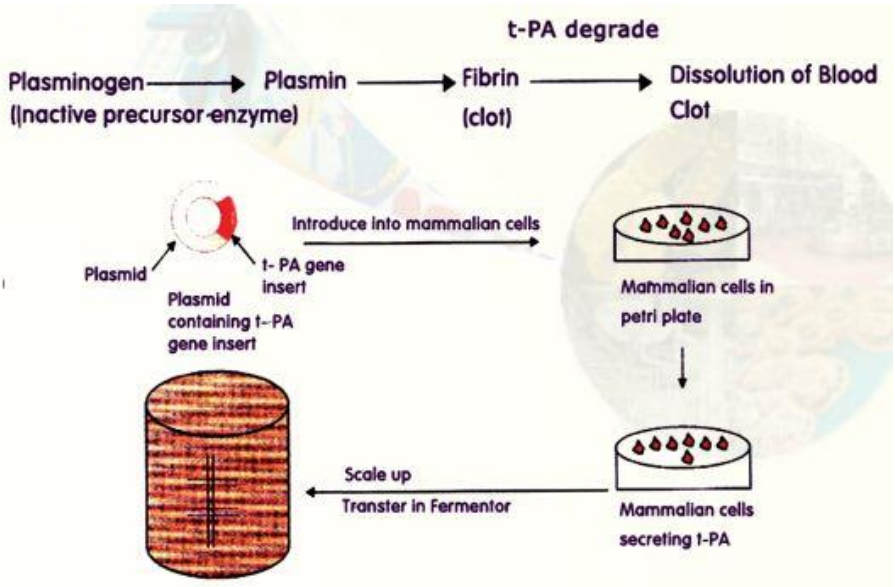


Fig. 6. Production and mode of action of tPA.

48) **EPO- Erythropoietin** is a hormone like substance released by the kidney in response to hypoxia (shortage of oxygen) or anoxia (lack of oxygen).

49) The **use of r-HuEPO is advantageous** over blood transfusion as it does not require donors or transfusion facilities, and the risk of transfusion associated disease is minimised

50) HuEPO- recombinant human erythropoietin has been produced using **Chinese Hamster ovary (CHO) cell lines** and it's effectively used in the treatment **of anemia, renal failure etc.**

51) Like EPO, **factor VIII** is also a glycoprotein and is required for blood clotting. Used to treat Haemophilia A. Factor IX is used to treat **Haemophilia B**

52) Antibodies bind to specific domains of macromolecules (antigens) known as **epitope**s.

53) **Monoclonal antibodies** are produced by **Hybridoma technology.**

54) Hybridomas are obtained by **fusing an antibody producing lymphocytes cell and a Myeloma cel**l. (A spleen cell of mouse immunized with red blood cells from sheep)

55) **Myeloma cell** - bone marrow tumor cell

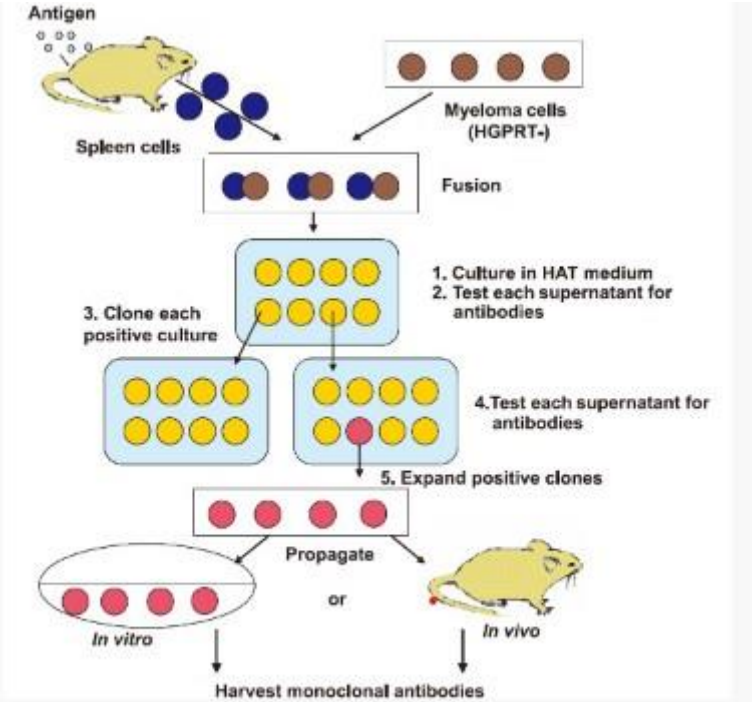


Fig 2. mAb generation by the hybridoma technology

56) **Polyclonal Antisera** are derived from many cells and therefore they are heterogeneous antibodies that are specific for several epitopes on an antigen.

57) Generally monoclonal antibody **OKT 3** is used for the treatment of acute renal allograft rejection.

58) OKT–3 was the first monoclonal antibody to be approved for the treatment of **acute rejection in clinics**

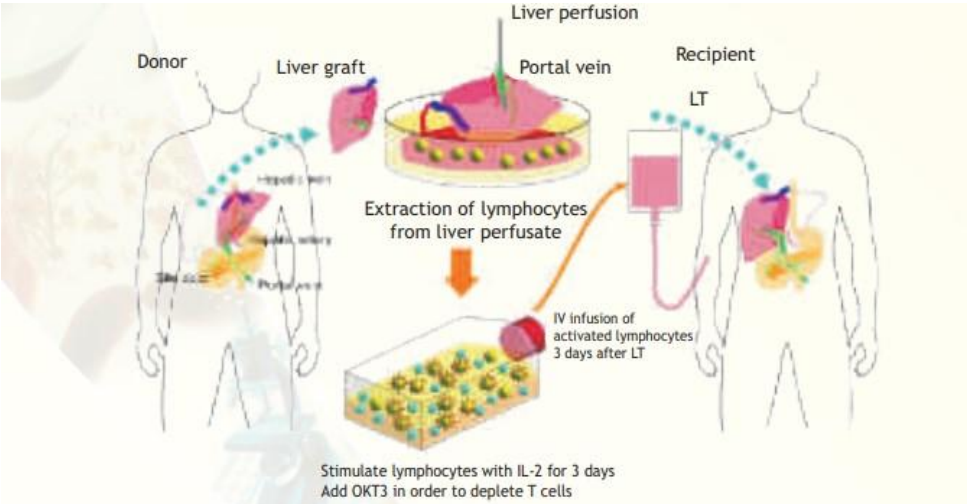


Fig. 8. Adoptive immunotherapy with OKT3 during liver transplantation (LT).

59) **T-cells** play an important role in rejecting foreign tissue in transplants so patient needs suitable treatment to suppress T cells. OKT3 is hence highly effective therapeutic agent which reserves acute **renal allograft rejection**.

60) **Herceptin (trastuzumab**) is a monoclonal antibody approved for therapy of early-stage breast cancer that is Human Epidermal growth factor Receptor 2-positive (HER2+).

61) **Stem cells** are cells that retain the capacity to self-renew as well as produce progeny. They are more restricted in both mitotic potentials the range of distinct types, differentiated cells, which can give rise like skin, blood and intestinal epithelium and subject to continuous renewal throughout life and must maintain an adequate number of cells that retain the potential to proliferate to make good such losses.

62) The formation of blood cells is also called hematopoiesis.

63) **A typical blood cell formation** can be divided into **four stages**:

a. **Stem cell** which retains the capacity to self-renew.
b. **Progenitor cells** which have limited self-renewal capacity greater capacity to differentiate.
c. **Precursor cells** which have no capacity to self-renew or differentiated.
d. **Mature cells** which are those which neither have the capacity to renew or differentiate nor hence are completely differentiated.

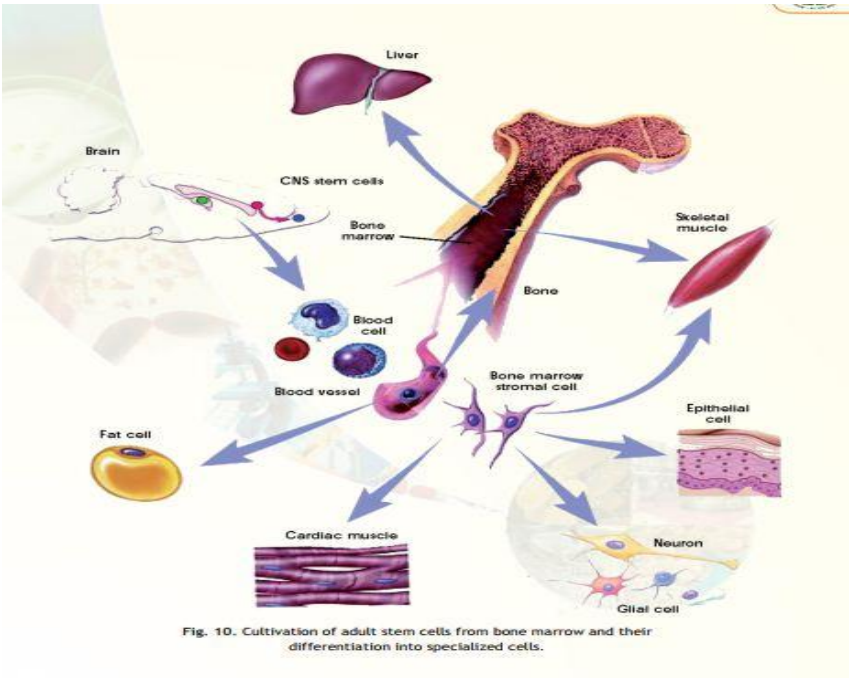
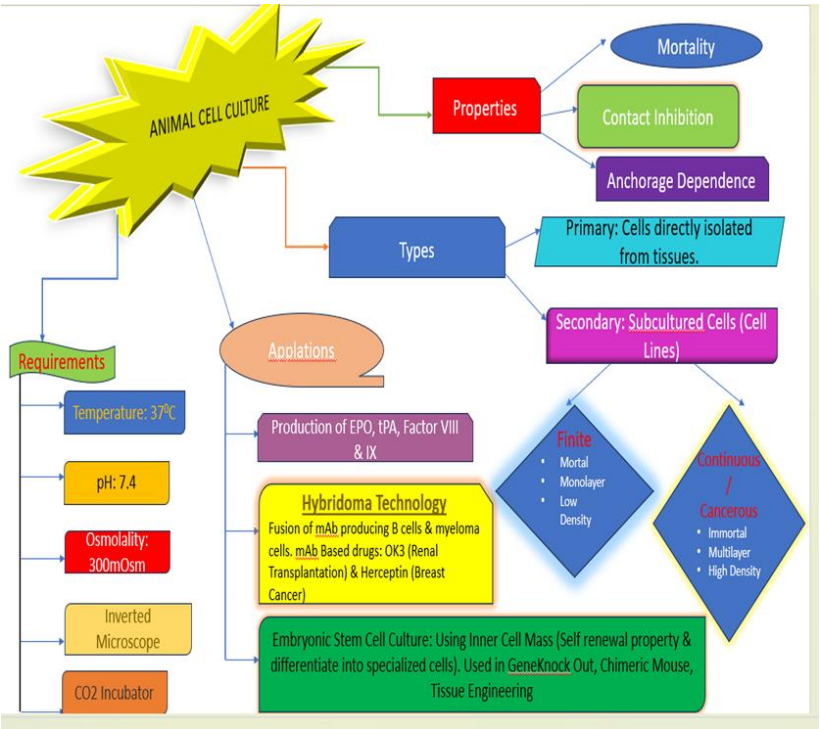


Fig. 10. Cultivation of adult stem cells from bone marrow and their differentiation into specialized cells.

64) **Embryonic stem cells** are cell lines desired directly from the inner cell mass of fertilized mouse embryo without any use of transforming agents.

65) The field of stem cell research was established in 1960s by **Ernest McCulloch and James T**ill at the University of Toronto.

MIND MAP



MULTIPLE CHOICE QUESTIONS

1. Name the type of culture which is prepared by inoculating directly from the tissue of an organism to culture media?
a) Primary cell culture
b) Secondary cell culture
c) Cell lines
d) Transformed cell culture

Ans : a) Primary cell culture

2. What is a cell line?
a) Multilayer culture
b) Transformed cells
c) Multiple growth of cells
d) Sub culturing of primary culture

An d) Sub culturing of primary culture

3. Which of the following is NOT the major function of the serum?
A) Promotion of tuber and bulb formation
b) Stimulate cell growth
c) Enhance cell attachment
d) Provide transport proteins

Answer a) Promotion of tuber and bulb formation

4. Which of the following is not a basic requirement for animal cell culture?
A. Sterile environment
b. Appropriate growth medium
c. Uncontrolled temperature and pH
d. Adequate oxygen and carbon dioxide levels
Answer: c. Uncontrolled temperature and pH

5. Which of the following is a common challenge in animal cell culture?
A. Optimizing growth conditions
b. Avoiding genetic drift
c. Minimizing contamination
d. All of the above
Answer: d. All of the above

6. Animal cell culture deals with
a. The isolation of cells from animal tissue
b. Surgical intervention for removal of tissues or organs from animal
c. Cells placement into an environment (media) in order to enhance their survival and proliferation

d. All of the above
Answer: d. All of the above

7. Essential requirements for growth of animal cells are
a. Regulated temperature

B. Proper substrate for an attachment of cells on appropriate growth medium

C. An incubator that maintains the correct ph and defined osmolality
d. All of the above

Answer: d. All of the above

8. Cell culture techniques has been used a stool in which areas

A. Molecular genetics
b. Immunological analysis and surgery
c. Bioengineering and pharmaceutical industry
d. All of the above
Answer: d. All of the above

9. Cell medium supports

A. Cell survival

B. Cell proliferation
c. Cellular functions
d. All of the above
Answer: d. All of the above

10.Which of the following is NOT the disadvantage of animal cell culture?
A) Small size (high sensitive techniques to detect changes).
B) Scale-up is challenging
c) May not represent in vivo phenotype/genotype
d) none of the above

Answer d. None of the above

11.Which monoclonal antibody approved for therapy of early-stage breast cancer?
A) Herceptin
b) OKT3
c) t-PA
d) none of the above

Answer a. Herceptin

12.Which of the following is NOT the disadvantage of animal cell culture?
A) Small size (high sensitive techniques to detect changes).
B) Scale-up is challenging
c) May not represent in vivo phenotype/genotype
d) none of the above

Answer d. None of the above

13.Haematopoesis is
a) formation of RBC
b) Formation of platelets
c) WBC formation
d) none of the above

Answer a. Formation of RBC

14.haematopoesis occurs in
a) spleen
b) bone marrow
c) both
d) none of the above

Answer c. Both

15.Which of the following is NOT the disadvantage of animal cell culture?
A) Small size (high sensitive techniques to detect changes).
B) Scale-up is challenging
c) May not represent in vivo phenotype/genotype
d) none of the above

Answer d. None of the above

16.Which tissues are subjected to continuous renewal?
A) blood
b) intestinal epithelium
c) skin
d) All of the above

Answer d. All of the above

17.Subculturing involves
a) removing the growth media
b) disassociating the adhered cells
c) diluting the cell suspension into fresh media
d) All of the above

Answer d. All of the above

18.Mode of growth is monolayer in
a) finite cell line
b) continuous cell line
c)both
d) none of the above

Answer a) finite cell line

19.Growth rate is rapid in
a) finite cell line
b) continuous cell line
c) both
d) none of the above

Answer b) continuous cell line

20.Which of the following is NOT true regarding sub culturing?
A) required to periodically provide fresh nutrients
b) Splitting of cells
c) needed to grow secondary cell line
d) form primary cell line

Answer d. Form primary cell line

21.Adherent cells are
a) derived from connective tissue
b) not mobile
c) Adhere to the vessel
d) All of the above

Answer d. All of the above

22.most commonly used enzymes for separating cells in cell culture
a) Pepsin.
B) trypsin and collagenase
c) Chymotrypsin
d) none of the above

Answer b. Trypsin and collagenase

23.Cancer cells
a) are rounded
b) pile up on each other
c) lose contact inhibition
d) none of the above

Answer d. All of the above

24.Which of the following is feature of animal cell culture?
A) Animal cells can be grown in glass or plastic vessels
b) They can be grown only for limited generations
c) they divide and fill the surface of the culture vessel and then stop growing
d) All of the above

Answer d. All of the above

25. When cells grow and reach the walls of the container (i.e., reach confluency) they stop growing further and this is termed as
a) Colony formation assay
b) transformation
c) infinite cell line
d) contact inhibition

Answer d. Contact inhibition

26. Homogenous population of cells derived from a single parental cell is called
a) Clone
b) Stem cell
c) Epitope
d) none of the above

Answer a. Clone

27.First cell line established is
a) HeLa
b) Hybridoma
c) CHO
d) none of the above

Answer a. Hela

28. Advantages of animal cell culture are
a) Homogenous genetic population.

b) Controlled physico-chemical environment
c) Easy to add genes (Transfection) or regulate protein levels

d) All of the above

Answer d. All of the above

29.Animal cell vulnerable to contamination due to
a) The growth rate of animal cells is relatively slow
b) usually require 18 to 24 hour to divide

c) a small number of bacteria would soon outgrow a larger population of animal cells.
d) All of the above

Answer d. All of the above

30.Stem cells have
a) capacity to renew
b) formed from ICM
c) differentiate
d) All of the above

Answer d. All of the above

31.Who amongst these were the first to introduce proteolytic enzyme trypsin for subculture of animal cells:

a) Kohler and Milstein
b) Rous and Jones
c) McCulloh and Till
d) Smith and Nathans

Answer b)Rous and Jones

32.Which of these is **NOT** a function performed by sodium bicarbonate in animal cell culture:

a) pH Maintenance
b) Acts as carbonate source
c) Maintains osmolality
d) Provides sterility

Answer d) Provides sterility

33.What is the need of a CO2 incubator in animal cell culture?

a) Maintains pH and Sterility
b) Maintains high relative humidity
c) Maintains high temperature
d) All of these

Answer d)All of these

34.Which of the following gene transfer methods in animal cells need HEPES buffered saline?

a) Lipofection
b) Microinjection
c) Electroporation
d) Calcium Phosphate mediated

Answer d) Calcium Phosphate mediated

35.Match the following and choose the correct option:

Proteins	**Therapeutic Use**
i) Factor VIII	A) Cancer Therapy
ii) FSH	B) Stroke
iii) T-PA	C) Infertility
iv) IL2	D) Haemophilia A

A. i -> B, ii -> A, iii -> D, iv -> C
B. i -> D, ii -> C, iii -> B, iv -> A
C. i -> C, ii -> D, iii -> A, iv -> B
D. i -> A, ii -> B, iii -> C, iv -> D

Answer b) i -> D, ii -> C, iii -> B, iv -> A

36.Which of the statements regarding inverted microscope is true?

a) Optical System is at the bottom while the light source is at the top.
b) Optical System is at the top while the light source is at the bottom.
c) Both Optical System and Light Source are at the top.
d) Both Optical System and Light Source are at the bottom.

Answer a) Optical System is at the bottom while the light source is at the top

37.Which of the properties of multipotent stem cells is **NOT** true?

a) They are lineage restricted

b) They are adult stems cells

c) They can differentiate into all types of specialized tissues.

d) They act as the repair system for the body.

Answer c) They can differentiate into all types of specialized tissues

38.What is the expansion of ICM that are used for embryonic stem cell culture?

a) Interior Cellular mass

b) Inner Cell Mass

c) Integrated Cell Mass

d) Inside Cellular Matrix

Answer b) Inner Cell Mass

39.What is the principle behind Gene-Knock Out?

a) Homologous Recombination

b) Homozygous Relocation

c) Homologous Relocation

d) Homozygous Recombination

Answer a) Homologous Recombination

40.Identify the correct order of sub culturing of animal cells in culture.

a) Washing the plate -> Removing growth media -> Dissociating adhered cells -> Diluting cell suspension into fresh media.

b) Removing growth media -> Diluting cell suspension into fresh media -> Dissociating adhered cells -> Washing the plate.

c) Diluting cell suspension into fresh media ->Washing the plate -> Dissociating adhered cells -> Removing growth media.

d) Removing growth media ->Washing the plate -> Dissociating adhered cells -> Diluting cell suspension into fresh media.

Answer d) Removing growth media ->Washing the plate -> Dissociating adhered cells -> Diluting cell suspension into fresh media.

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**: OKT 3 is used to prevent graft rejection following kidney transplantation
   Reason: OKT3 blocks immune cells which attack foreign grafts

   Answer: A

2. **Assertion**: CO2 incubators are required for animal cell culture
   Reason: Cells are grown in 5-10% CO2.

   Answer: A

3. **Assertion**: ru EPO is produced using CHO cell line
   Reason: it is more beneficial than blood transfusions for anemic patients.

   Answer: A

4. **Assertion**: OKT 3 is used to prevent graft rejection following kidney transplantation
   Reason: OKT3 blocks immune cells which attack foreign grafts

Answer: A

5. **Assertion**: HEPES buffer is really expensive
   Reason: it has superior buffering capacity in the ph range 7.2–7.4

Answer: B

6. **Assertion**: Selection of proper growth medium is most significant and critical stage for animal cell culture
   Reason: Appropriate media selection will depend on the kind of cells to be cultured and on the requirement for culture, such as growth, differentiation and production of desired products like pharmaceutical compounds.

Answer: B

7. **Assertion**: Antibiotics such as penicillin and streptomycin is used in culture media

Reason: Antibiotics are essential for growth of cells

Answer: B

8.Assertion: Some additional approaches are applied for the isolation of cell culture products

Reason: The serum in the culture media may interfere with the purification and isolation of cell culture products including pharmaceutical compounds

Answer: A

8. Assertion: Animal cell culture vulnerable to contamination

Reason: Growth rate of animal cells is relatively slow and usually requires 18 to 24 hours to divide.

Answer: A

10. **Assertion**: All cells within a clonal population are genetically identical

Reason: A homogenous population of cells can be derived from a single parental cell termed as a clone

Answer: A

VERY SHORT ANSWER QUESTIONS

1. Which serum is always added in the culture medium of animal cell culture?

Ans: Fetal calf serum.

2. Which filter sterilizes the LAF Hood

Ans: High Efficiency Particulate Air (HEPA) is used to sterile the chamber.

3. What is the difference between monoclonal antibodies and polyclonal antibodies?

Ans: Monoclonal antibodies bind very specifically to an epitope on an antigen. Polyclonal antisera on the other hand are derived from many cells and contain heterogeneous antibodies that are specific for several epitopes on an antigen.

4. Write down any two features of animal cell growth in culture.

Ans: Animal cells can grow in simple glass and plastic containers in nutritive media but they grow to only limited generations. Animal cells exhibit contact inhibitions and cell to cell interaction.

5. What are stem cells?

Ans: Stem cells are cells that retain the capacity to self-renew as well produce progeny that are more restricted in both mitotic potentials the range of distinct types of differentiated cells they give rise to.

6. How recombinant vaccines are developed to stimulate immunity against specific diseases?

Ans: Recombinant vaccines are developed on the basis of selected epitopes, provide immunity against specific disease. (Antibodies bind to specific domains of macromolecules (antigen), are called epitopes)

7. How can we distinguish the normal animal cells and the cancerous cells?

Ans: We can distinguish the normal cells and the cancerous cells by observing the shape of the cancerous cells. Cancerous cells are more rounded and they lose contact inhibition.

8. Write the name of the pH indicator which is used in animal cell culture.

Ans: Phenol red.

9. Name the clot lysing drug which is produced mammalian cell culture?

Ans: Tissue Plasminogen Activator (t-PA)

10. What is the osmolality of the commercial media?

Ans: The final osmolality of all commercial media is around 300m Osm.

11. How can we observe the animal cells which are developed in situ during the tissue culture in petri plate?

Ans: By inverted microscope we can observe the cells of the petri plate.

12. what are the properties associated with the continuous cell line?

Ans: Properties are: the ploidy (change in basic number of chromosomes), no contact inhibition and no anchorage dependence.

13.What is growth rate and doubling time of primary culture??

Ans: The growth rate is slow and doubling time is around 24 to 96 hours.

14. Why is maintaining pH important in animal cell culture?

Ans: pH is important for maintaining the appropriate ion balance but also for maintaining optimal function of cellular enzymes and for optimal binding of hormones and growth factors to cell surface receptors.

15. What is role of bicarbonate source in the animal cell culture?

Ans: The bicarbonate content of the medium neutralizes the effect of increased CO2.

SHORT ANSWER QUESTIONS

1.. Define the following-

(I) Sub culturing (Il) Adherent cell (III) Suspension culture

Ans: (i) Sub culturing: Sub culturing involves removing the growth media, washing the plate, disassociating the adhered cells, usually enzymatically (e.g. with trypsin), although some cells may be removed by repeated pipetting or gentle scraping), and diluting the cell suspension into fresh media.

(ii) Adherent cells: Cells which grow adhering to cell culture vessel are adherent dependent are called adherent cells.

(ii) Adherent cells: Cells which grow adhering to cell culture vessel and are adherent dependent are called adherent cells.

(iii) Suspension Cultures: Cells which do not attach to the surface of the culture vessel and grow in a suspended manner in the culture medium are called suspension cultures.

2.What is the importance of pH while culturing animal cells? How is pH maintained?

Ans: Regulation of extra-cellular and intra-cellular pH is essential for survival of individual mammalian cells. Most media maintain the pH between 7 and 7.4.

The pH is important for: -

i. Maintaining the appropriate ion balance and optimum function of cellular enzymes.

ii. Optimal binding of hormones and growth factors to cell surface.

The pH is regulated through buffering system. Most media use a bicarbonate- CO_2 system.

3.. What are the advantages of using established cell lines for research by the scientists?

Ans: The advantages of using established cell lines is that their growth characteristics, media requirements and responses to selected reagents are established and therefore convenient for the scientist to use.

4. What determines the osmolality of the media used for culturing of cells?

Ans: The osmolality of the medium used is determined by the media formulation. Salt and glucose are the major contributors to the osmolality of the medium, although amino acids may also contribute significantly Osmolality is checked directly with an osmometer.

5.a) What is EPO? What is it used for?

b) Why is it advantageous over transfusion?

Ans:a) EPO is recombinant human erythropoietin (r-HuEPO) produced using Chinese Hamster ovary cell lines. R-HuEPO is virtually identifiable endogenous hormone.

It is effective in the treatment of anemia associated with surgery, AIDS, Cancer chemotherapy and chronic renal failure.

b) The use of r-HuEPO is advantageous over blood transfusion as it does require donors, transfusion facilities etc. There is no risk of disease, to patient receiving the blood.

6.What are the inner mass cells? What are their characteristics?

Ans: If we fertilize a mouse egg and grow it in tissue culture (in vitro fertilization), it is observed that after several steps of cleavage, the cell which are dividing get accumulated in one corner. These cells are called inner cell mass (ICM) cells. These cells would be maintained in tissue culture in the presence of Irradiated fibroblast cells. It was observed these cells:

i. Could retain the characteristics of the embryo founder cells, e after prolonged culture and extensive manipulation.

ii. Were able to reintegrate fully into embryogenesis when regenerate into the embryo.

ili. Could be used to create chimeric mice.

iv. Could maintain a stable euploid karyotype.

v. Could self-renew without differentiating in culture.

7. Distinguish between Finite cell lines and continuous cell lines?

Ans: (a) Finite cell lines: 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 dependency. The mode of growth is the monolayer form. The growth rate is slow and doubling time is around 24 to 96 hrs.

(b) Continuous cell lines: Cell lines transformed under in vitro culture conditions give rise to continuous cell lines. The various properties associated with continuous cell lines are:

- The ploidy is either aneuploid or heteroploid,
- There is no contact inhibition and no anchorage dependency.
- The mode of growth is either monolayer or suspension form.
- The growth rate is rapid and doubling time is 12 to 24 hrs.

8. Which monoclonal antibody is sued in therapy pf early breast cancer what is HER2+? How does it work in the therapy of cancer?

Ans Trastuzumab is a monoclonal antibody approved for therapy of early-stage breast cancer that is Human Epidermal growth factor

Receptor 2-positive (HER2+). Cancer cells overexpress HER2+ along with dysregulation of receptor activation. Trastuzumab works by attaching itself to HER2 receptors that receive signals which in turn stimulate the cells to proliferate and blocking them from receiving growth signals. The result is impaired growth of breast cancer

9. What are the applications of animal cell culture?

Animal cell culture has diverse applications in various areas. Some are listed below:

• As a model system for the study of interaction between cells and diseases causing agents as well as their drugs

• As a convenient and economic tool to study virus research

• As a useful technique for vaccine production on a large scale

• As a production house for various medically important protein pharmacueticals

10. How does OKT3 prevent transplantation rejection?

Therapeutic MAB – OKT3 Muromonab-CD3 (OKT-3) is a murine monoclonal antibody directed against CD3 receptor

It is used as an immunosuppressant drug that is given intravenously to reverse acute rejection of transplanted organs, such as kidney, heart and liver.

When OKT–3 is bound to CD3, the T-cell receptor (TCR) undergoes endocytosis resulting in an inert T-cell and acts by blocking the function of T cells which play a major role in acute graft rejection This prevents subsequent recognition. T cells are then eliminated by phagocytosis. After OKT–3 therapies are over, T cell function usually returns to normal within a week

11.. Fill in appropriately the pharmaceutical hormones, cells used to culture them and their use.

	Pharmaceutical protein	Cells used	Therapeutic use
1	Follicle Stimulating Hormone	CHO cells	----------
2	----------	CHO cells	Anemia
3	Factor IX	CHO cells	----------
4	----------	CHO cells	Stroke
5	Monoclonal antibodies	----------	Cancer therapy & Autoimmune diseases
6	----------	CHO cells	Hemophilia A

12.1 ans) infertility

12.2 ans) Erythropoietin

12.3 ans) Hemophilia B

12.4 ans) Tissue Plasminogen Activator (tPA)

12.5 ans) Hybridoma cells

12.6 ans) Factor VIII

LONG ANSWER TYPE QUESTIONS

1. What are the physical factors that affect the cells growing under in vitro conditions?

Ans: The physical factors that affect the cells growing under in vitro conditions are temperature, pH, osmolality, gaseous environment, supporting surface and protection of cells from chemical, physical and mechanical stresses. Cell cultures are grown in incubators maintained at 37°C. Most mammalian cell cultures are grown in incubators maintained at 37°C because it is the core body temperature of Homo sapiens. Most

media strive to achieve and maintain the pH between 7 and 7.4. Different cell types may have an optimum pH slightly outside this range. The regulation of pH is done through a variety of buffering systems.

The osmolality of the medium used is determined by the media formulation.

Salt and glucose are the major contributors to the osmolality of the medium, although amino acids may also contribute significantly. Almost all commercial media are formulated to have final osmolality of around 300 mOsm.

The composition of the media also plays a crucial role in the success of cell culture because the medium provides the essential nutrition that are incorporated into dividing cells, such as amino acids, fatty acids, sugar ions, trace elements, vitamins, cofactors and ion necessary to maintain the proper chemical environment for the cell.

Serum is another component of animal cell culture because it contains growth factors which promote cell proliferation, cell attachment and adhesion factors.

2.What do you mean by the term Hybridoma technology?

Ans: Antibodies obtained from serum are a heterogeneous population of protein released by different populations of B-lymphocytes and therefore are known as polyclonal antibodies bind very specifically to an epitope on an antigen. They are produced from antigen activated B lymphocyte cells that have been immortalized by hybridizing (fusing) them with a myeloma cell (cancerous lymphocyte). Ceasar Milstein and George Kohler developed the immortalized hybridoma cell line by fusion of antibody producing B cells and cancerous cell (myeloma) in presence of polyethylene glycol (PEG). Thus the hybrid retains the ability of the B cells to secrete antibodies and the ability of the myeloma cell to grow indefinitely. The Hybridoma technology has created a revolution in the area of diagnostics and antibody based therapy.

3.a) why is serum one of the most important component of cell culture media?

b) What are the disadvantages of serum?

Ans) a) Serum is one of the most important components of cell culture media because

- Serum is considered a good source for amino acids, proteins, vitamins, carbohydrates, lipids, hormones, growth factors, etc.
- Serum provides several binding proteins, like albumin, transferrin, which can carry other molecules into the cell.
- In addition, serum also supplements adhesion factors that help the cells to adhere to substratum before they begin to divide

b) There are certain disadvantages of serum in media:

• Serum contains insufficient amount of cell specific growth factors and thus, cannot be used as standalone for culture.

• It may also contain cytotoxic compounds and some growth inhibiting factors that will cause the inhibition of the cultured cell growth and proliferation

. • Serum may contain high risk of contaminants, like various virus, fungi and mycoplasma.

• The serum in the culture media may interfere with the purification and isolation of cell culture products including pharmaceutical compounds and thus, some additional approaches are applied for the isolation of cell culture products

4. How is regulation of pH achieved in animal cell culture?

Ans} For optimum culture conditions, the regulation of pH is critical and is achieved by one of the following buffering systems:

(A**) Natural buffering system**: In a natural buffering system, gaseous CO2 balances with the CO2/HCO3 content of the culture medium. The concentrations of CO2, HCO3 and pH are interrelated. By increasing the external/exogenous CO2, the pH will be reduced making the medium acidic. The culture media with a natural buffering system need to be maintained in air with 5 –10 percent CO2, which is usually maintained by a CO2 incubator. Natural buffering system is low cost and non-toxic. The presence of pyruvate in the medium results in the increased endogenous production of CO2 by the cells. Thus, the use of pyruvate is advantageous because of less dependency on the exogenous supply of CO

Bicarbonate buffering follows Le Chatelier's principle. Increased acidity in the medium is established by an increase in Hydrogen (H+) ions; free bicarbonate ions then react with the extra H+ ions to form carbonic acid 'shifting the reaction to the left', stabilizing pH. Similarly, a decrease in H+ ions will result in a 'shift to the right'. CO3 - + H+ H2CO3 - Increase

H+ caused acidity Decrease H+ caused reduced acidity Stabilis tion of pH over a range of % CO_2 can be achieved using an appropriate HCO_3^- concentration

(B) **Chemical buffering system** In the chemical buffering system, a zwitter ion, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), is used that has a superior buffering capacity in the pH range 7.2–7.4, and with HEPES, a controlled gaseous atmosphere is not required. It is relatively expensive and toxic at a higher concentration for some cell types.

5. Name and Describe the various equipments required for animal cell culture.

Ans: Equipment Required for Animal Cell Culture are

a. Laminar Air Flow Chambers (LAF) or Tissue Culture Hoods.

b. CO_2 Incubators

c. Inverted microscope

d. Cell counter

e. Centrifuge

a. LAF: Animal cell culture work involves the risk of infection with pathogenic organisms, hence all experimental work of inoculation of media and cell manipulation must be done in aseptic conditions. The pathogenic forms of bacteria and fungi spoil the culture as they grow much faster. For maintaining an aseptic environment, Laminar Air Flow (LAF) cabinet provides a clean workbench for culture manipulators. While working with the LAF cabinet, the worker has to use an apron so that he or she is protected from any contamination and at the same time the culture is protected from any contamination from the worker. LAFs may be classified into three types depending upon the nature of cells or organisms. Each cabinet is housed in an area where workers do not frequent much. At a time, two workers can operate for cell manipulation.

Class I: These are horizontal workbenches in which air is drawn from the front passing through the culture of cells, and flows out through the top of the hood. The LAF is housed in a protected area that is sterile so that the air sucked inside is also sterile. The lab space surrounding the hood is frequently disinfected. The workers have to wear aprons and slippers

meant for the purpose so that they are protected from any contamination.

Class II: These types of LAF s are a bit specialized in which the filtered air is drawn

Class III: These are specialized cabinets to work with hazardous materials (radioisotopes, carcinogenic or toxic drugs) and highly pathogenic organisms. These are biohazard hoods, which should be used preferably by one person at a time. It should be easily cleaned from inside as well as below the workbench in the event of any spillage. The front screen is made of Perspex that should be raised, lowered or removed to facilitate cleaning and handling bulky culture apparatus. The workers have to use special protective clothing, sterilized gloves and have to be screened periodically for checking contamination and radiations

b) CO2 Incubators: The CO, incubator is a specialized instrument, which has devices to maintain proper CO2 tension, constant temperature and sterile conditions.

Blowing air over a humidifying tray controls the relative humidity inside the chamber and mixes O2 and CO, in the correct ratio to control CO2 tension.

By controlling the gas phase, the inside of the incubator maintains a congenial atmosphere for the growth of animal cells and tissues.

The CO2 incubators are expensive and use HEPA filters to allow highly purified air to maintain the sterile environment of the incubator. Highly purified air is to maintain the sterile environment of the incubator.

c. Inverted Microscope: Inverted microscope is required to observe morphological changes in the cultured cells. When the culture of cells is placed in a Petri dish they remain at the bottom while the medium remains above them. Hence an ordinary microscope is unable to view them.

Inverted microscope has a specialized optical system having the light source at the top and the optical system at the bottom. The microscope stage is big enough so as to hold big roller bottles. With improved visibility one can observe the living culture of cells, growth patterns and also the patterns of any microbial infection. The living cultures can also be photographed by an inverted microscope that remains above them so that we can view them.

c) Inverted microscope has a specialized optical system having the light source at the top and the optical system at the bottom. The microscope stage is big enough so as to hold big roller bottles. With improved visibility one can observe the living culture of cells, growth patterns and also the patterns of any microbial infection. The living cultures can also be photographed by inverted microscope.

d) Cell Counter: A cell counter is required to study the quantitative growth kinetics. It is more helpful when two or three cell lines are cultured. The cell counting can be done on a glass slide with a counting chamber held under a microscope. The most accurate device for counting is the coulter counter.

e). Centrifuge: Low speed centrifuges are used for separating cells. Operating temperatures are kept low, i.e. around 20°C and for this purpose the centrifuges are operated in temperature- controlled room. Exposure of cells to higher temperatures is avoided.

CASE BASED QUESTIONS

1. 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 multi cellular 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 case of mouse that haematopoiesis occurs in the spleen and bone marrow. In human being 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 1960s by **Ernest McCulloch** and **James Till** at the University of Toronto.

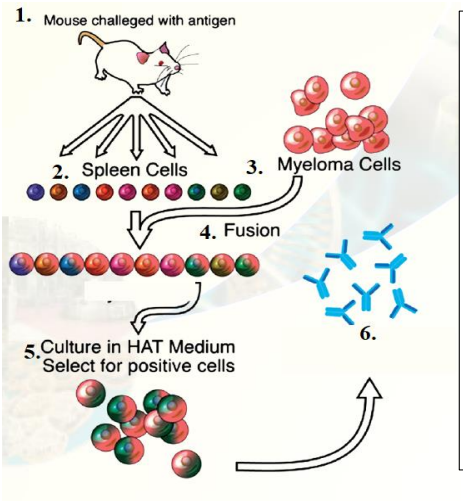
A. What is depicted in the above given image?
B. Name two diseases which can be treated with stem cell therapy.
C. Write two sources of stem cells in an adult human being
D. Differentiate between pluripotent stem cell and a multi potent stem cell.

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

Q2. 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 continuos fashion
4. Monoclonal antibody

Q3. 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.

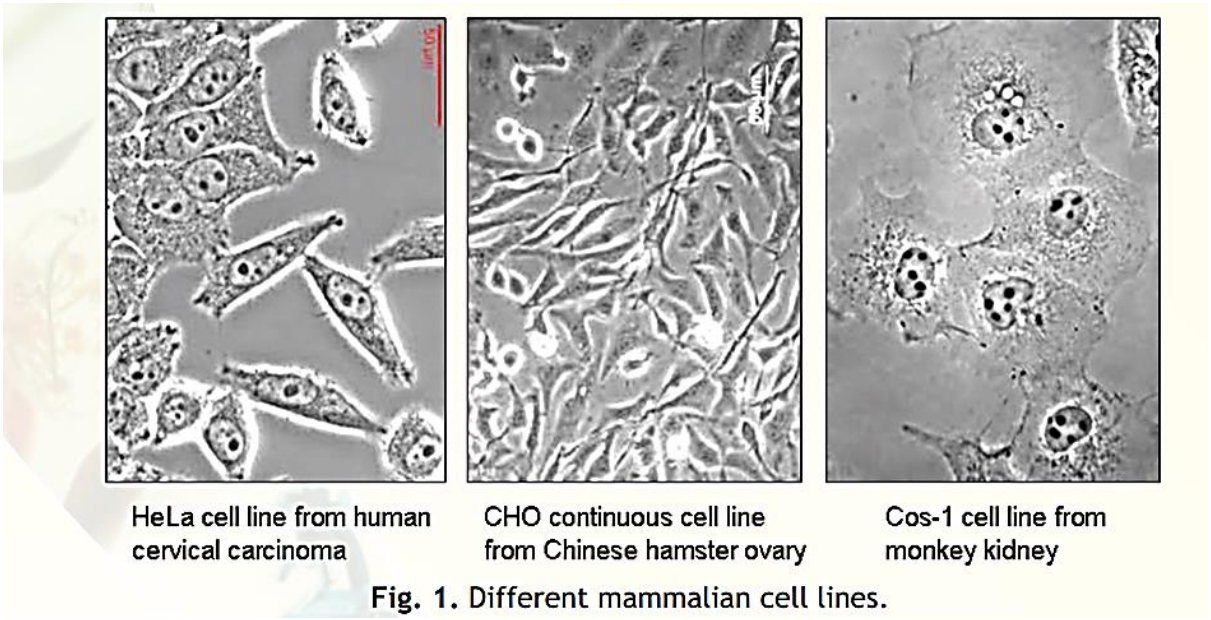


Fig. 1. Different mammalian cell lines.

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 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. Continuos 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

Q4. 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

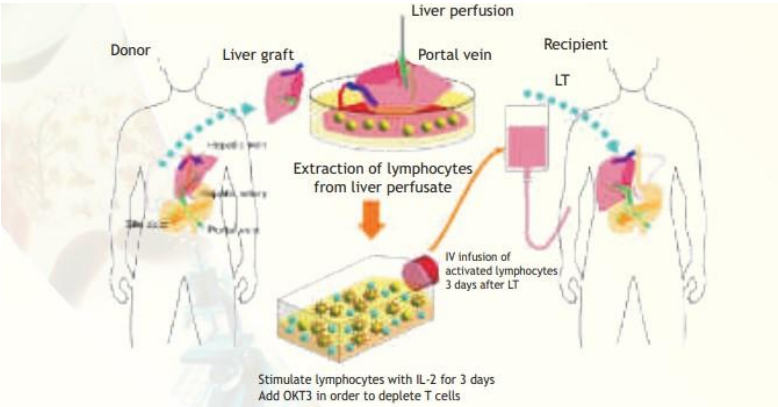


Fig. 8. Adoptive immunotherapy with OKT3 during liver transplantation (LT).

1.Which monoclonal antibody is immunosuppressant?

2. Which organs are generally transplanted in case of organ failure?

3.How does OKT3 acts?

4. Which cells act as first line of defense in our body?

Answers

1.OKT3 is 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.

Q5. 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.

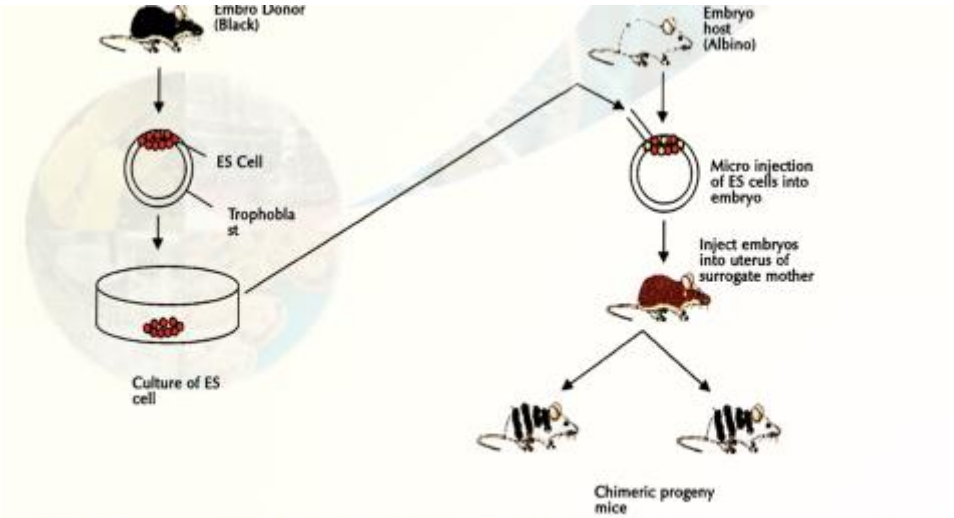


Fig. 11. Creation of chimeric mice using ES cells

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

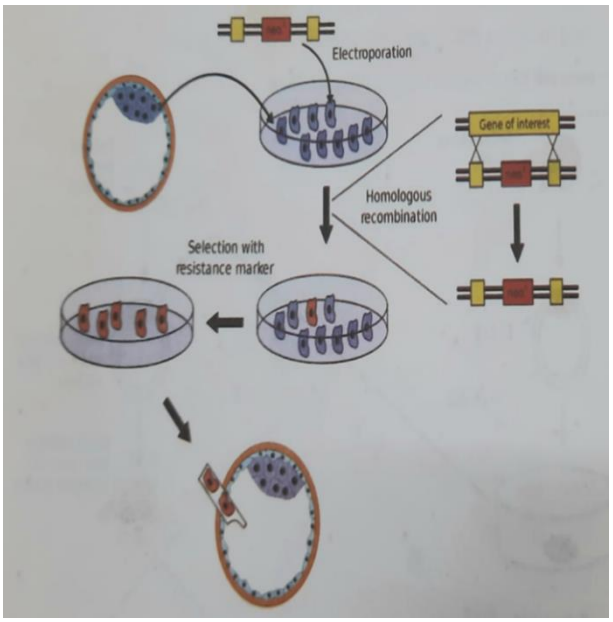
1. The ES cells are cell lines derived directly from the inner cell mass of growing embryos without use of immortalizing or transforming agents
2. The inner cell mass (lCM) of embryos can be maintained in cell culture in the presence of irradiated fibroblast cells
3. The stem cells:

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

1.



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

MEDIA LINKS

1. https://www.youtube.com/watch?v=_udMO7nlNpo&list=UUEXFE-gL9GydVqFZ-7favWQ&index=15 (part1)
2. https://www.youtube.com/watch?v=k2SDRUnuVvg&list=UUEXFE-gL9GydVqFZ-7favWQ&index=14 (part 2)
3. https://www.youtube.com/watch?v=CxuBLaTIGnE&list=UUEXFE-gL9GydVqFZ-7favWQ&index=13 (part 3)
4. https://www.youtube.com/watch?v=w936wbss-5A&list=UUEXFE-gL9GydVqFZ-7favWQ&index=7
   (part 4)
5. https://www.youtube.com/watch?v=FQK_78t-uCM&list=UUEXFE-gL9GydVqFZ-7favWQ&index=6 (part 5)
6. https://www.youtube.com/watch?v=w936wbss-5A&list=UUEXFE-gL9GydVqFZ-7favWQ&index=7
7. https://www.youtube.com/watch?v=p78b0koN69g
8. https://www.youtube.com/watch?v=o0hHD2yjjNI
9. https://www.youtube.com/watch?v=8vnpmRANztU

Question Papers

SOLVED BOARD QUESTION PAPER
BIOTECHNOLOGY (045) Class XII (2023-24)

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*

(B) *Bartonella henselae*

(C) *Bacillus amyloliquefaciens*

(D) *Barnesville coli*

2. Severe combined immunodeficiency disease is caused due to the absence of :
1

(A) Adenosine diphosphate

(B) Adenosine deaminase

(C) Adenosine cyclase

(D) Guanidine nitrate

3. Single nucleotide polymorphisms usually occur in _regions. *1*

(A) Coding

(B) Non-coding

(C) Regulatory

(D) Exonic

4. Artificial seeds are produced by encapsulating the somatic embryos at the _____stage in a protective coating. *1*

(A) Torpedo

(B) Globular

(C) Cotyledon

(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

(B) Antibiotics

(C) Blood serum

(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*

(B) YEp

(C) pBR322

(D) M13

7. An example of secondary metabolites produced

by microbial cells include *1*

(A) Vitamins

(B) Alcohol

(C) Acids

(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

(B) Gene pollution

(C) DNA contamination

(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*

(B) *Penicillium chrysogenum*

(C) *Penicillium eutrophus*

(D) *Penicillium cerevisiae*

12. _____cultures can be maintained for a prolonged period of time by repeated sub-culturing.

1

(A) Ovary

(B) Protoplast

(C) Callus

(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 human genome.

Reason (R) : Unreliability of in silico gene prediction is responsible for reporting lesser number of genes in 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 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 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 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*

OR

(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 cause 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.

(ii) Name the computer programmes that can perform gene prediction for bacterial and eukaryotic genomes.

MARKING SCHEME
BIOTECHNOLOGY (045)
(2023-24)

SECTION – A

Sl. No.	Value Poin	Marks
1	(C) Bacillus amyloliquefaciens	1
2	(B) Adenosine deaminase	1
3	(B) Non-coding **/** (A) Coding (As per the prescribed text book, pg63,both options(A)and(B) are correct)	1
4	(A) Torpedo	1
5	(C) Blood serum	1
6	(D) M13	1
7	(D) Antibiotics	1
8	(B) Gene pollution	1
9	(A) 2001	1
10	(B) Pluripotent	1
11	(B) *Penicillium chrysogenum*	1
12	(C) Callus	1
13	(A) Both Assertion (A) and Reason (R) are true and Reason (R) is the correct explanation of the Assertion (A) .	1
14	(D) Assertion (A) is false, but Reason (R) is true.	1

15 (B) Both Assertion (A) and Reason (R) are true, but the Reason (R) is ***not*** the correct explanation of the Assertion (A). 1

16 (C) Assertion (A) is true, but Reason (R) is false. 1

SECTION – B

17 2

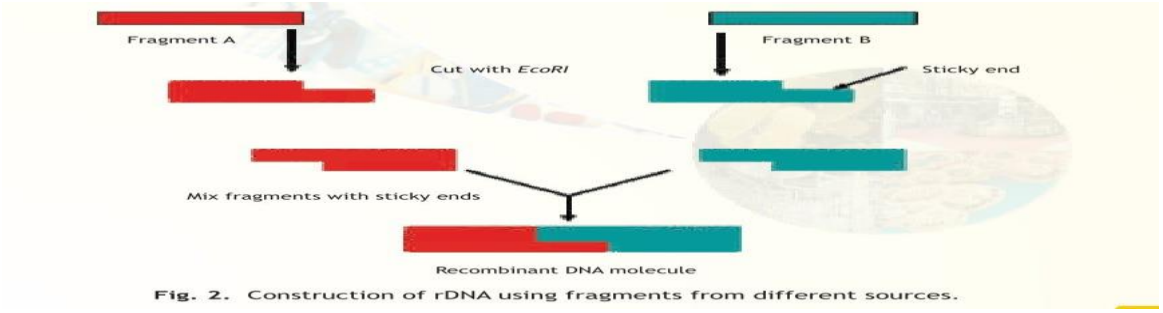


Fig. 2. Construction of rDNA using fragments from different sources.

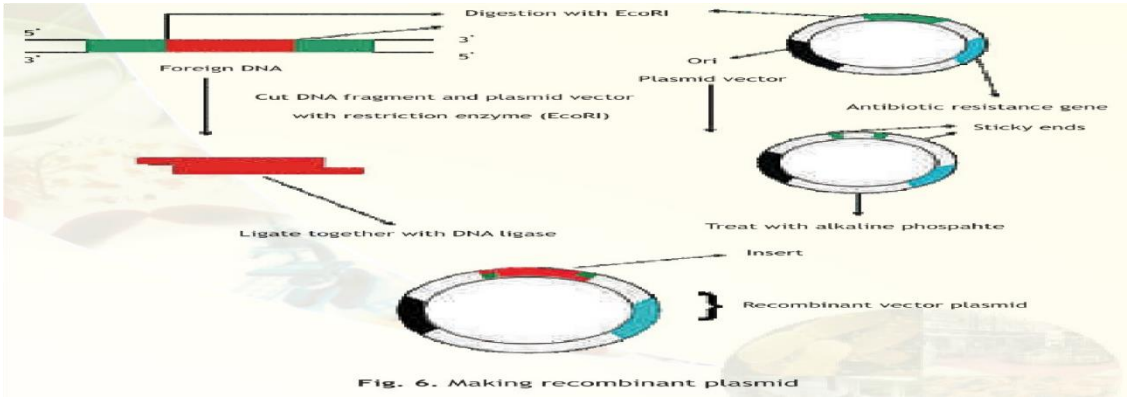


Fig. 6. Making recombinant plasmid

18

To understand the genetic basis of a disease
To search for new diagnostic methods 1+1=
To search for new therapeutic modality or uses (Any two) 2

19 Two dimensional Gel Electrophoresis Technique / Mass Spectrometry Technique 1+1=2

Principle of Two dimensional Gel Electrophoresis Technique:
Separation of proteins is on the basis of charge and size. First proteins get separated on the basis of isoelectric pH (pI) by IEF technique and then on the basis of molecular size by SDS PAGE technique .

Principle of Mass Spectrometry Technique:
It determines the molecular weight of a chemical compound or protein by separating the
molecular ions according to m/z ratio.

(Any one technique with principle)

20 -To overexpress a gene that encodes for the first enzyme in the biosynthetic pathway
-To use Agrobacterium rhizogenes to induce excessive secondary roots (hairy roots) in plants that normally produce useful secondary metabolites in this region. 1+1=2

21 (a) Drawbacks : Small size / Scale up is challenging / may not represent in vivo phenotype or genotype 1+1=2
(Any two for 1 mark each)

OR

(b) (i) The oncologist is trying to determine whether the tumour is cancerous or not. 1
(ii)Cell comprising tissues and organs like liver of an animal grow only to a certain size after which they cease to grow / Infant animal grow only to adulthood and not any further. +1=2

SECTION C

22 The principle involved in FISH technique is hybridization of DNA of metaphase chromosomes affixed to a microscopic slide with a fluorescent DNA probe. 1

The principle of Microarray is that complementary sequences will bind to each other by base pairing or hybridisation. Fluorescently labelled single stranded probe binds with single stranded DNA molecule spotted on the microarray plate. 1

Applications of FISH are :

Diagnosis of genetic diseases

Locating specific DNA sequences

Identification of presence or absence of a gene

To study translocation of genes on chromosomes. (Any 1 point) ½

Applications of Microarray are :

To monitor the whole genome on a single chip for interactions among thousands of genes simultaneously.

To compare the amounts of many different mRNA in two cell populations in tissue specific genes to study the regulatory gene defects, cellular response to environment, cell cycle variations. (Any 1 point) ½

OR

(b)

	Expression Proteomics	Functional Proteomics
1	Study of qualitative and quantitative expression of proteins in different environment or disease.	Identification and analysis of protein networks involved in a living cell/ nuclear pore complex/ study of protein functions and interactions/ molecular mechanisms and biological roles.
2	Used to identify disease specific proteins	To analyse the properties of molecular networks involved in a living cell.
3	To provide understanding of the basis of tumour development.	Identification of novel proteins which are important for translocating important molecules from cytoplasm of a cell to nucleus and vice versa.

½ x 6 =3

.

S. No.	Protein Pharmaceutical	Therapeutic Use	Animal Cell Line
(A)	Erythropoietin	Anaemia	CHO cell line
(B)	Herceptin	Breast cancer therapy	CHO cell line
(C)	Interleukin 2	Cancer therapy	CHO cell line
(D)	Tissue plasminogen activator	Stroke	CHO cell line

½ x 6 =3

Any Three

24 Step 1 –

Denaturation : The DNA duplex gets separated at temperature above 80°C to form two single stranded DNA templates.

½ x 6= 3

Step 2 –

Annealing : Two primers bind to the 3' end of DNA templates at temperature between 50 - 60°C

Step 3 –

Extension : Each primer is extended by Taq DNA polymerase in 5' 3' direction using dNTPs and the DNA strand as template at 70°C

25 A – Trypsin

B – Paper Electrophoresis

C – Sequencing

1 x 3 = 3

26 Viable plate count method [colony forming units(CFU)]– counting the number of live cells Turbidity measurement – Absorbance at a particular wavelength is proportional to cell concentration ½ x 6 =3

Coulter counter – Direct counting of cells in suspension as they pass through electrical field in a single file.

Dry weight – to measure constant weight of fixed volume of culture after drying.

Wet weight- to measure weight of fixed volume of culture.

ATP measurement- to measure ATP in the beginning end at the end of the culture.

[Any Three ways with explanation]

27 Inactive, harmless precursors of proteolytic enzymes are called zymogens. 1

Sl. No.	Chymotrypsinogen	Chymotrypsin
1	It is inactive precursor of chymotrypsin enzyme.	It is fully active enzyme.
2	The substrate-binding pocket is blocked/ not exposed.	The substrate-binding pocket is not blocked and is exposed.
3	Serine 195 is not acidic.	Serine 195 is acidic.
4	Charge relay doesn't operate	Charge relay operates

1+1 = 2

Any two points

28. Ampicillin resistance gene -- provides ampicillin resistance ½ x 6 =3

Lac Z gene -- produces β galactosidase enzyme

GFP gene – Produces Green Fluorescent Protein

Tetracycline resistance gene -----provides tetracycline resistance

Leu 2 gene -- codes for an enzyme needed for synthesis of amino acid leucine

Any three

29. (a) Protein A is extracellular — 1

(b) Solvent extraction / Chromatography — 1

(c) Fig 10, Pg. 100 — 2

OR

(c) Lesser number of steps for downstream processing are advisable for :

(d) Less cost

(e) High yield

30. (a) Sucrose — 1

(b) Inositol — 1

(c) Auxins and Cytokinins — 2

1+1=2

OR

(b) - Autoclaving: Sterilisation is performed at 15 pounds per square inch pressure for 20 minutes in an autoclave

- Membrane filter sterilisation- Culture medium is forced through a membrane of very fine pore size.

31. (a) (i) Elevation of glutathione (a reducing compound) in cells that detoxifies xenobiotics. — 1+1=2

Protects cellular components from oxygen intermediates and free radicals. — 1+1=2

) Jaundice / Infected skin lesions / genito urinary tract infections / Intestinal infections. (iii) Curd is used as a probiotic as it is a source of beneficial bacteria which can colonise the intestinal tract. — 1

OR — 3

(b) (i) Recombinant vaccine based on selected epitope: Synthetic gene for an epitope of a virus is assembled

and introduced into host cells which are grown on large scale . The epitope protein is isolated, purified and used as recombinant or subunit vaccine. 1+1=2

) Thermal stability / pH stability / Solvent tolerance / Solubility / Catalytic potency/

Biological adaptation to environmental stresses such as high salinity, drought , cold, etc. Any two

32. a) i) ddNTPs lack 3′Hydroxyl group so the phosphodiester bond between 3' hydroxyl group of the previous nucleotide cannot be formed with the 5' phosphate group of the incoming nucleotide and hence the growing DNA chain cannot be further extended and the chain gets terminated. 2 3

(ii) The sequencing technique is carried out in four test tubes, each carrying single stranded DNA template, deoxy nucleotide tri phosphates, primer and DNA polymerase. A small amount of four dideoxy nucleotide triphosphates i.e. ddATP, ddTTP, ddGTP and ddCTP are added separately into the four test tubes and the reaction is allowed to proceed. Prematurely terminated strands in a given tube are separated on special gels by electrophoresis wherein the bands can be resolved even if they differ by one nucleotide . The shorter fragments move faster towards the anode. The radioactive primers help in easy visualisation using autoradiography. The gel is read from bottom to top to arrive at 5′ to 3′ original DNA sequence. 2

OR

(b) (i) - The fastest moving shortest DNA fragment is obtained at 5' position (towards anode). 1+1=2

- Since DNA synthesis occurs in 5' to 3' direction, the gel is read from 5' end (anode)

(ii) Single tube DNA sequencing uses fluorescent colours rather than radioactive isotopes so is **safer.** 1

It is **better** as it is automated /faster /uses a single lane gel for electrophoresis/ result is directly displayed on a computer screen and data can be stored in a computer.

(iii) M13-based vector

33.

(a) (i) **Entrez** allows us to access literature in the form of abstracts , sequences and structures. Entrez provides comprehensive information on a given biological question. ½ x 6= 3

Taxonomy browser provides information on taxonomic classification of various species.

Locus link provides information on official gene names, descriptive information about genes and on homologous genes

(ii) **UniProtKB** gives information about annotated protein sequences. 1+1 =2

PDB (Protein Database) contains information about three dimensional structure of proteins.

OR

(b) (i)In BLAST: 1 x 3 = 3

- A given sequence is compared with the database sequences using matrices which give scores. They either reward a match or penalise a mismatch.
- Top scoring matches are ranked based on whether the match was due to ancestral relationship or just a random chance.
- True matches are examined through ENTREZ

(ii) GeneMark for bacterial genomes and 1+1= 2

UNSOLVED SAMPLE PAPER I
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

1. Which of the following sequences will be digested by restriction endonuclease?
   a. CCGCAATCGATC
   b. ATTCGGCCTGCT
   c. ATGCGCTAAACC
   d. GCTTCGATGGGC
2. Which of the following RE will produce a blunt end?
   a. *Eco*RI
   b. *BamHI*
   c. *Hind*III
   d. *Alu*I
3. Which of the following order is correct in respect to the insert size of the vectors?
   a. Plasmid< λ-phage < Cosmid < BAC <YAC
   b. Plasmid> λ-phage > Cosmid> YAC> BAC
   c. Plasmid< λ-phage < BAC < YAC < Cosmid
   d. Plasmid > Cosmid > λ-phage > BAC> YAC
4. Which of the following is the correct order of steps in peptide mapping?
   a. Trypsin treatment → Purification of protein → paper electrophoresis → paper chromatography
   b. Purification of protein → trypsin treatment → paper chromatography → paper electrophoresis

c. Purification of protein → trypsin treatment → paper electrophoresis → paper chromatography
d. Purification of protein → paper electrophoresis → trypsin treatment → paper chromatography

5. Which of the following statements are not true about organophosphates?
   a. They react with acidic serine residues of enzymes
   b. They have been used as mosquito repellents
   c. Nerve gas which is a serine alkylating agent knocks off the activity of acetyl choline esterase
   d. These compounds increase the rate of reaction of acidic serine residue
6. Which of the following is the correct decreasing order of PER value of proteins?
   a. Whey, milk, casein, soya, rice, wheat
   b. Milk, whey, casein, soya, rice, wheat
   c. Wheat, milk, casein, soya, rice, whey
   d. Wheat, rice, soya, casein, milk, whey
7. Which of the following mutations causes cystic fibrosis in humans?
   a. Deletion of 3bps resulting in loss of codon 508
   b. Increased number of CAG repeats more that 35 times
   c. Deletion of 3bps resulting in loss of codon 308
   d. Single base change in *ApoE* gene
8. Which of the following is not covered under proteomics?
   a. Characterization of entire protein complement
   b. Sequencing the whole genome
   c. Study of protein-protein interactions
   d. Protein function and localization
9. Which of the following is not correct pair regarding microbial products?
   a. *Aspergillus niger* – Citric acid
   b. *Aspergillus oryzae* – Vitamin B12
   c. *Alcaligenes eutrophus* - PHB
   d. *Escherichia coli* - Insulin
10. Which of the following is the correct sequence of Plant Tissue Culture?
   a. Explant→ sterilization → transfer to medium → incubation →organogenesis → hardening

b. Explant→ transfer to medium → sterilization → incubation →organogenesis → hardening
c. Explant→ sterilization → transfer to medium → organogenesis → incubation → hardening
d. Explant→ sterilization → transfer to medium → incubation → hardening →organogenesis

11. CO_2 incubator is used in animal cell culture. Which of the following statements is not true?
a. It is designed to maintain high CO_2 concentration with constant temperature
b. It is designed to maintain high CO_2 concentration with high relative humidity
c. It is designed to maintain high CO_2 concentration with sterile condition
d. It is designed to maintain high CO concentration with O_2 concentration

12. You have found a bacterium to grows at temperatures about 50^o C. What will you do to grow it in your laboratory?
a. Provide microbial growth medium and incubate at 40^o C
b. Provide animal growth medium and incubate at 50^o C
c. Provide microbial growth medium and incubate at 50^o C
d. Provide any growth medium and incubate it at room temperature

Question No. 13 to 16 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

13. **Assertion**- LAF protects the tissue culture from the operator.
Reason- LAF protects the operator from the tissue culture.

14. **Assertion**- Calcium alginate is used in artificial seeds.
Reason- The somatic embryos are encapsulated in a protective coating.

15. **Assertion**- M13 phage-based vector in DNA sequencing.

Reason- M13 phage-based vector can exist as a single stranded replicative form.

16. **Assertion**- Proteolytic enzymes like trypsin and chymotrypsin are stored in their zymogen form.
Reason- Both trypsin and chymotrypsin are serine proteases.

SECTION B

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

18. Why whey protein can be used to treat numerous ailments? [2]

OR

Why a sports person is given BCAA before and after exercise?

19. Mention the use of the following in microbial cell culture: [2]
    a. Aeration
    b. Agar
    c. Antifoams
    d. Corn-steep liquor

20. What is 2-D gel electrophoresis? Why is it better than one directional gel electrophoresis? [2]

21. Identify the database based on the information available: [2]
    a. Three-dimensional structure of proteins
    b. Cluster of all ESTs of a single gene
    c. Curated database of mRNA and proteins
    d. Nucleotide sequence

SECTION C

22. What is Single nucleotide polymorphism? With the help of any two examples explain the relevance of studying SNPs. [3]

23. Why subtilisin is used in detergents? Why do we need to improve this enzyme? How is subtilisin improved to be used in detergents? [3]

24. What is primary cell culture? Outline the steps in Primary cell culture. Mention the disadvantages primary cell culture.
[3]

25. How is primary metabolite different from secondary metabolite? Also mention the examples of both.
[3]

26. How is dNTP different from ddNTP? Write the ddATP product for the DNA sequence 3'- ATGCGGTCGA-5.
[2+1]

27. Why there is a need of two primers in PCR? Why is there is need for thermostable DNA Polymerase? If a sample has 5 molecules, after 25 cycles how many molecules will be produced?
[1+1+1]

28. What is BLAST? What is the principle involved in BLAST?
[3]

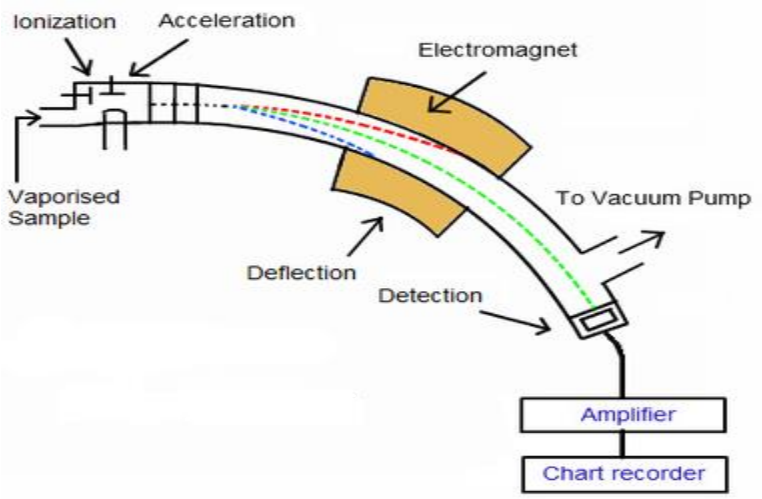
OR

How is FISH used to study the effectiveness of anti-cancer drug on CML patients? Draw diagram to show reciprocal translocation.

SECTION D [Case-based question of 4 marks each]

29. Mass Spectrometry

Mass spectrometry has emerged as an important tool in biotechnology. It is extremely useful in obtaining protein structural information such as peptide mass or amino acid sequences. It is also useful in identifying the type and location of amino acid modification within proteins.



a. A sample with molecular weight M greater than 1200 Da can give rise to multiple charged ions. Write down the general

formula for calculating the mass of the charged molecule with n no. of H^+.

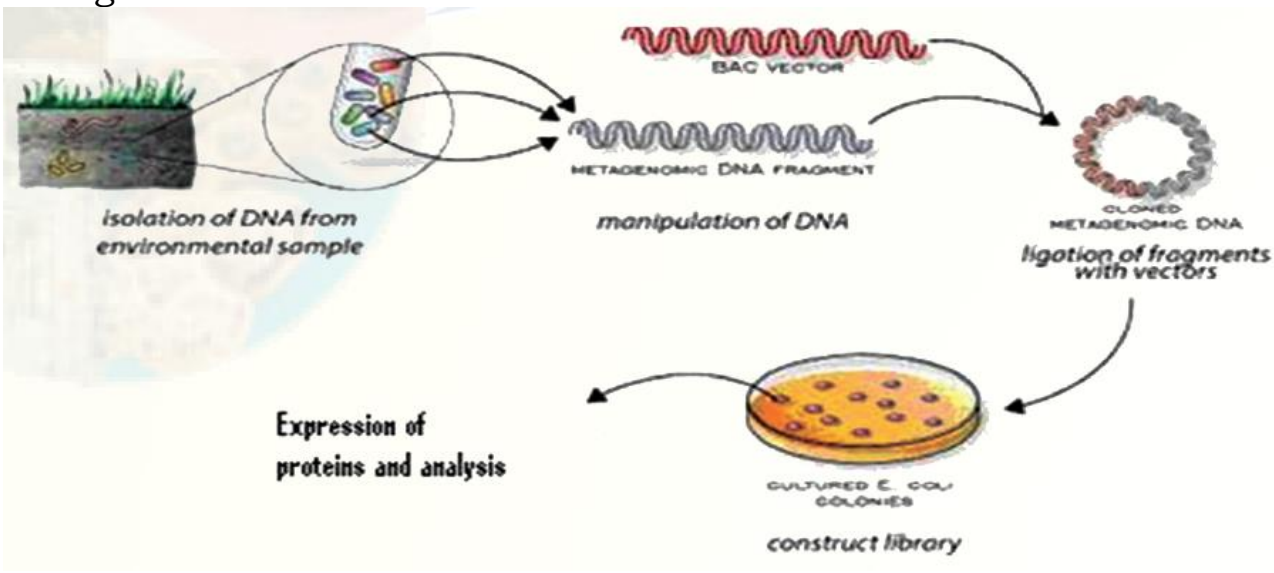
b. What is the advantage of mass spectrometry?

c. How is ion formation of ions and sample volatilization achieved in mass spectrometry?

OR

A polypeptide which has molecular mass 50000 Da. It was charged +1, +2, +3 and +4. Calculate the m/z ratio for all four ions.

30. The following schematic diagram shows the strategy for metagenomics-



a. Why is metagenome?

b. What is the advantage of studying metagenome over genome of one organism?

c. You have found a novel antibiotic gene in a bacterium from soil. But the bacteria cannot be cultured in laboratory. How can you produce this antibiotic on industrial scale?

OR

After DNA extraction how will you construct the library? Draw a flow chart to show the steps involved.

SECTION E

31. What is Embryonic Stem (ES) cell? Where are these cells derived from? How are they maintained in culture? Mention any 2 properties of ES cells. [5]

OR

How is secondary cell culture of animal cells done? Draw a flow chart to show the steps of secondary cell culture. How are cell lines established?

32. (i) Why was crop of rice modified in golden rice? How is golden rice made rich in Vitamin A.

(ii) You want to improve seed protein quality of a cereal. How will you ensure that the amino acids are expressed only in the seed?

(iii) Name the genes you will introduce to prepare genetically modify crop and give an example of GM Crop with the same resistance-

a. *Helicoverpa armigera* resistant
b. Virus infection

[2+1+2]

OR

What is micropropagation? How is micropropagation different from vegetative propagation? Mention the sequence of steps of micropropagation. Cite any two advantages of micropropagation.

[5]

33. a. Why do we need to use selection methods to identify the recombinant host cells?

b. Which selection methods will you use to select host cells when pUC 19 is used? Why?

c. What is the principle behind the various selection methods? Briefly explain it.

[1+2+2]

OR

a. What is the importance of ori site in a vector?
b. Why is the presence of MCS beneficial over unique restriction endonuclease in a vector? Name a vector that contains MCS. Also name the selectable marker gene of this vector contains MCS.
c. What is a shuttle vector? How is it different from a vector like pUC? [1+2+2]

****

UNSOLVED SAMPLE PAPER II
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

1. In small intestine, the protein digesting enzymes synthesized are 1

   a) Trypsin and HCl

   b) Chymotrypsin and HCl

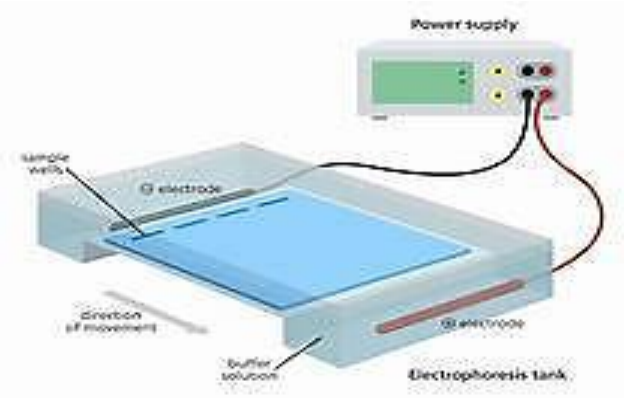
   c) Trypsin and Chymotrypsin

   d) Papain

2. Male sterility is widely used in crops such as maize, sunflower for hybrid production. Male sterile plants are created by introducing a gene encoding- 1
   (a) Barnase protein
   (b) TA29
   (c) Barstar protein
   (d) Coat protein
3. In Foreign DNA is directly introduced into the recipient cell using a fine micro-syringe to transform it. The probable advantage this provides could be: 1
   a) No specialized equipment required
   b) No damage to cells
   c) Low transduction rate

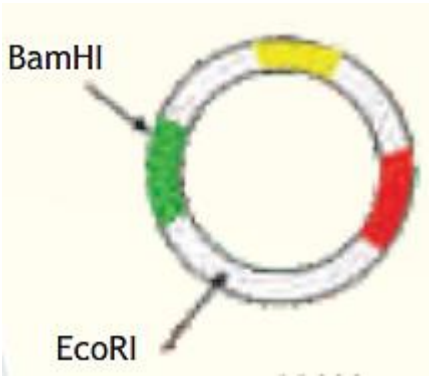
d) Precision of delivery

4. The given diagram is showing



a) Isolation of DNA molecules
b) Cutting of DNA into fragments
c) separation of DNA fragments according to their size
d) Construction of recombinant DNA by joining with cloning

5. Identify the type of vector 1



A.Expression vector B. Ti Plasmid C. Shuttle Vector D. pBR322

6. Being a researcher, you want to improve the deficiency of certain amino acids in cereals and legumes. Choose the technique out of the following which will be the best to achieve your goal: 1

a. Plant tissue culture
b. Adding fertilizers to soil
c. Protein engineering
d. Vegetative Propagation

7. Which of the following is cultured to obtain virus free plants? 1

(a) Embryo

(b) Nucleus

(c) Apical bud

(d) Anther

8. A 23 Kb DNA fragment has to be cloned in a host cell. Which vector should be used for this experiment?
1

a) Plasmid
b) Cosmid
c) BAC
d) Bacteriophage lambda

9. Chymotrypsin hydrolyses peptide bonds following –
1

A. bulky aromatic amino acids

B. Hydrophobic amino acids

C. Basic amino acids

D. Polar amino acids

10. Native enzyme Subtilisin is inactivated by bleach in detergents because of oxidation of methionine at position 222. Choose a strategy that will help overcome this problem:
1

a) Use Pepsin instead of Subtilisin
b) Eliminate use of bleach
c) Substitute another amino acid at position 222
d) Substitute another amino acid at position 221

11. The specific sequences of amino acids in the protein which stimulate immune response are known as ——
1

A. Epitopes

B. Sub unit vaccine

C. Muscle proteins

D. Recombinant vaccine

12. A piece of young hypocotyl was cultured in MS medium in a plant tissue culture lab. This is a type of-
1

(a) Organ culture

(b) Callus culture

(c) Explant culture

(d) Mass cell culture

Question No. 13 to 16 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

13. Assertion- Even if you know where the genes are in the genome, it is still difficult to count them.
Reason- Presence of over-lapping genes and splice variants makes the precise counting difficult.

14. Assertion**:** *Pichia* is preferred over *E. coli* for the expression of eukaryotic proteins.
Reason: Recombinant proteins cannot be easily obtained from *E. coli* cultures grown in fermenters.

15. **Assertion**: 2-D gel electrophoresis gives better resolution of results of protein analysis.
Reason: It involves two techniques – IEF and SDS-PAGE.

16. **Assertion:** RFLP stands for restricted filament length polymorphism

Reason – This indicates variation in size (length) of the restriction enzyme generated fragments among individuals belonging to two different species .

Section B

17. If you are growing animal cells in an incubator used for growing bacterial cells, do you expect the animal cells to grow? If not, why? 1+1=2

18. a. BCAA is important for muscle growth and activity. Why?

b. Expand BV and PER.
1+1=2

19. Explain two databases for gene level sequence of NCBI with information available on them.
2

20. Briefly explain the strategies in making the following traits in transgenic plants. 2
(i) Herbicide tolerant plants
(ii) Golden rice

21. Why is pH an important parameter during culturing of animal cells in culture? Which buffer system is used to maintain pH in animal cell cultures? 2

Section C

22. Give a schematic representation about generation of RFLPs. What is the principle behind the generation of RFLPs?
2+1=3

23. Enlist three essential features of cloning vector
3

24. i. Which tool of NCBI helps in the alignment of sequences in database?
ii. Diagrammatically show the difference in Philadelphia chromosome compared to normal in CML patient after performing FISH.
1+2=3

25. Normally the hydroxyl group of a serine residue is not acidic, but Ser 195 of chymotrypsin becomes acidic. How and why? Name any other serine protease.
2+1=3

26. i. A person suffering from chronic renal failure is intravenously injected with
r-HuEPO. Explain the mode of action of the injected EPO in the patient's body.
ii. Fill in the blanks: 1+2=3

Sl. No.	Proteins	Animal cell line used	Therapeutic Use
1	Follicle Stimulating Hormone	CHO	
2		CHO	Cancer Therapy

27. a) How are haploid plants is produced?

b) Name a chemical that is used to encapsulate embryos to prevent desiccation.

c) Name the plants used to create first inter species somatic hybrid. .1+1+1=3

28. Define SNP. Write any two diseases which are due to Single base differences and also mention their cause.

1+2=3

Or

Explain how comparative hybridization is carried out using microarray with suitable example

Section D

29. Strain isolation procedure described above only identifies a strain, which has the capability or potential to produce a desired molecule. It does not ensure that it produces molecule in sufficient quantities to be economically viable. Techniques of classical genetics and genetic engineering are used to improve the desirable characteristics of the strain. Mutation Selection: This is one of the oldest methods of strain improvement. Genetic Engineering Techniques: Until the recent breakthroughs in the techniques of genetic engineering, a bacterium could produce only substances coded for in its genome.

i. Name one mutagen.
ii. List two methods of strain isolation
iii. Name any two methods two improve strain quality.

1+2+1=4

30. **Peptide mass fingerprinting** (**PMF**) (also known as **protein fingerprinting**) is an analytical technique for protein identification in which the unknown protein of interest is first cleaved into smaller peptides, whose absolute masses can be accurately measured with a mass spectrometer such as MALDI-TOF or ESI-TOF. Then the proteins are cut into several fragments using proteolytic enzymes such as trypsin, chymotrypsin or Glu-C. A typical sample: protease ratio is 50:1. The proteolysis is typically carried out overnight and the resulting peptides are extracted with acetonitrile and dried under vacuum. The peptides are then dissolved in a small amount of distilled water or further concentrated and purified and are ready for mass spectrometric analysis.

a) Give names of two protein chemists scientist.

b) What is the mutation in sickle cell hemoglobin.
1

c) The order of steps occurring in protein fingerprinting is-
1

d) Why SDS-PAGE is considered a good technique for protein characterization.
1

Section E

31. a) What is doubling time? Write the equation for calculating doubling time.

b) If a bacterial culture contains 10^2 cells per ml at t_o and 10^7 cells per ml after 5 hours. Calculate its specific growth rate and doubling time. 2+3=5

Or

a) Draw the flowchart of downstream processing of an intracellular product produced during microbial fermentation at industrial scale

b) Enlist the various steps involved in gene cloning.
3+2=5

32.

a) Draw a labelled diagram to show the formation of a chimeric mouse.

b) In one experiment, a chimeric mouse is used as a model to study the function of a gene. Identify the technique.

c) Mention any one property of ES cell.
3+1+1=5

33. a) A group of students are trying to isolate recombinant insulin. After processing the fermentation broth, they observed no yield. What could be the most possible reason for this?

b) A recently discovered microbial strain gives us the desired metabolite in nanomolar concentration. Suggest two ways of improving the production of the desired metabolite.

c) *Pichia pastoris* has many advantages as a eukaryotic expression host. Justify giving two reasons. 1+2+2=5

****

UNSOLVED SAMPLE PAPER III
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

1. The osmolality of all commercial media is formulated to have around _____ and can be checked with _____.
   a. 300 mOsm, osmometer
   b. 250mOsm, osmometer
   c. 400 mOsm, pH meter
   d. 300 mOsm, pH meter

2. Which of the following molecules will you use to produce a eukaryotic protein in *E. coli*?
   a. Complementary DNA
   b. Genomic DNA
   c. mRNA
   d. Double stranded RNA

3. Which of the following diseases is not caused due to mutated protein?
   i. SCID ii. Sickle cell anaemia iii. Mad cow disease iv. Malaria
   a. i and ii
   b. ii and iii
   c. i and iv
   d. ii and iv

4. Which of the following is not true about the functional properties of whey proteins?
   a. Browning is caused by Millard reaction in which amino groups of proteins react with ketones of sugars

b. Water binding is done by hydrogen bonding of water in cakes and breads
c. Emulsification is done by stabilization of fat emulsions in salad dressings, soups, etc
d. Viscosity is achieved by thickening and water binding in soups and gravies, etc

5. The term 'Genomics' was coined by-
   a. H Winkler
   b. Craig Venter
   c. Margret Dayhoff
   d. Thomas Roder

6. In silico gene prediction is not an accurate way to calculate the number of genes as-
   I. Due to the existence of overlapping gene.
   II. There are multiple splice variants
   III. There is no direct correlation between the complexity of organism and the number of gene.
   IV. Human genome has 20 000 to 25 000 genes.
   a. I, II & III
   b. II, III & IV
   c. I, II & IV
   d. I, III & IV

7. Calculate the number of generations of a bacterial population in which the bacterial increases from 10^4 cells/ml to 10^8 cells/ml during four of exponential growth.
   a. 8 generations
   b. 10 generations
   c. 13 generations
   d. 15 generations

8. Which of the following is a bacterial toxin that is used to engineer crops resistant to bollworms?
   a. Bt-toxin
   b. Coat protein
   c. Thioesterase
   d. Acetolactate

9. Which of the following is not correct regarding virus free plants generated from virus infected plants?
   a. Virus free plants are used to increase yield and quality
   b. Apical and Axillary meristems are used to prepare virus free plants
   c. Virus free plants are resistant to viruses
   d. Vegetative propagules that are infected by virus are saved by micropropagation of meristematic tissue

10. You want to grow human blood cells in cell culture. What kind of culture can be done with blood cells?
   a. Adherent cell culture
   b. Suspension culture
   c. Colony formation assay
   d. Continuous cell culture

11. We use inverted microscopes in animal cell culture because-
    a. The culture vessels are deep so the medium is obstruction
    b. Animal cell are adherent so they grow on the bottom of culture vessels
    c. Observation of cell gives an immediate idea about the health of the culture
    d. Inverted microscopes have optical system at the bottom and light on top
12. Which of the following is not a microbial culture collection centre?
    a. ATCC
    b. DSM
    c. MTCC
    d. NCBI

Question No. 13 to 16 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

13. **Assertion**- Cryoprotectants are used in storing cultures for indefinite time.
    Reason- DMSO, glycerol, proline and mannitol are used as cryoprotectants in PTC.
14. **Assertion**- Gene knock outs are generated by making precise genetic modifications.
    Reason- Gene knockout mouse models are generated to study human diseases.
15. **Assertion**- DNA probes are tagged with fluorescent labels for hybridization experiments
    Reason- Fluorescent labels on DNA can be located with UV light
16. **Assertion**- Recombinant vaccines are safer than traditional vaccines
    Reason- Traditional vaccines may cause diseases due to incomplete inactivation of pathogens

SECTION B

17. In an experiment with pUC19 vector, the gene of interest was inserted and introduces in E. coli. The transformed host cells were then cultured in selection medium. The following observations were made regarding the colonies- [2]

Colony	Colour
I	Blue
II	White

a. Why do the host cells show blue and white colonies?
b. Which colony will you select to test for the expression of the insert gene

18. Why whey protein considered superior to all other sources of protein? [2]

OR

Mention the applications of the following protein-based products-

a. Hexokinase
b. Horseradish peroxidase
c. Chymosin
d. Glucose isomerase

19. Draw an outline of mass spectrometer. Expand MALDI and mention the use of MALDI in MS. [2]

a. Cancer cell and normal cell were compared with microarray technology. Cancer cell cDNA was labelled red and normal cell cDNA was labelled green. On scanning the microarray chip the following was observed-

Gene	A	B	C	D	E	F	G
Colour observed	Green	Red	Yellow	No Colour	Red	Yellow	No Colour

b. Which genes are expressed only in cancer cell?
c. Which genes are expressed only in normal cell?
d. Which genes are expressed in both cells?
e. Which genes are not expressed in either of the cells?

20. Mention any 2 analyses that can be made using bioinformatics tools. [2]

SECTION C

21. Briefly explain any three methods of introducing rDNA into host cells. [3]

22. Why are proteins designed? Give an example to illustrate protein engineering with an example. [3]

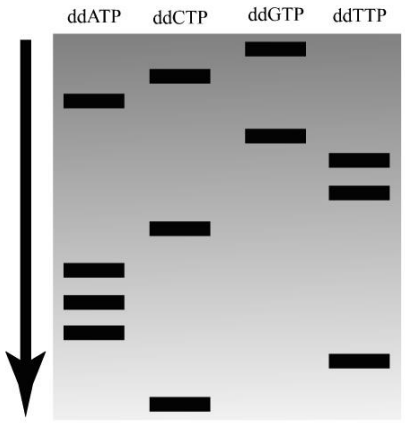
23. Mention any three applications of microbial cell culture. [3]

24. What is the principle of DNA fingerprinting? How is DNA finger printing done? (Draw a flowchart to show the steps) [3]

OR

a. How is single tube sequencing different from Sanger method of DNA sequencing?

b. Read the autoradiogram of Sanger method of DNA sequencing and arrive at the sequence of the template DNA.



25. Distinguish between finite an infinite cell line. Can we generate continuous cell lines from cancer cells? Name one such cell line. [3]

26. What are edible vaccines? Which crops will you use to produce edible vaccines for

a. human and

b. for animals [2+1]

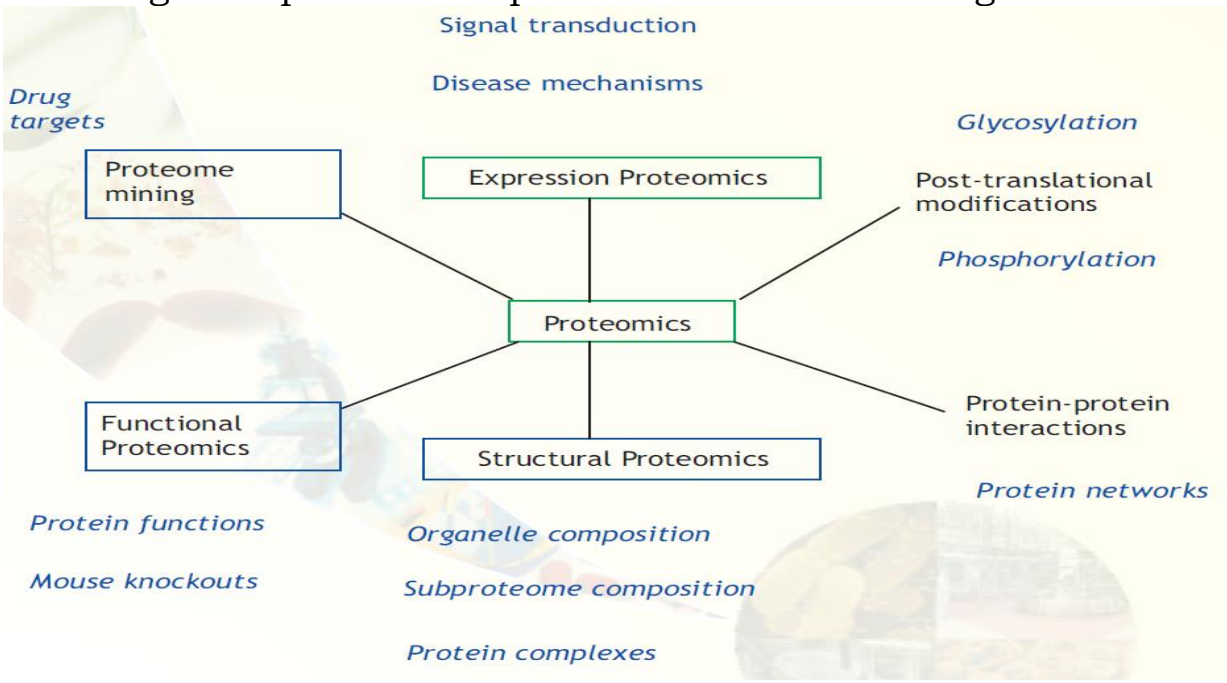
27. Mention the use of the following products of animal cell culture- [2+1]

a. R-HuEPO
b. Factor VIII
c. Factor IX
d. tPA
e. OKT3
f. Herceptin

SECTION D [Case-based question of 4 marks each]

28. Proteome

The proteome of a given cell is dynamic. In response to internal and external cues biochemical machinery of the cell could be modulated. This could lead to several changes in the proteins such as post-translational modifications, changes in cellular localization, effect on their synthesis or degradation. Thus, examination of a proteome is like taking a snapshot of the protein environment at a given time.



a. Why do we study the proteome of a cell?
b. What is proteome? What is proteomics?
c. How is structural proteomics different from expression proteomics?

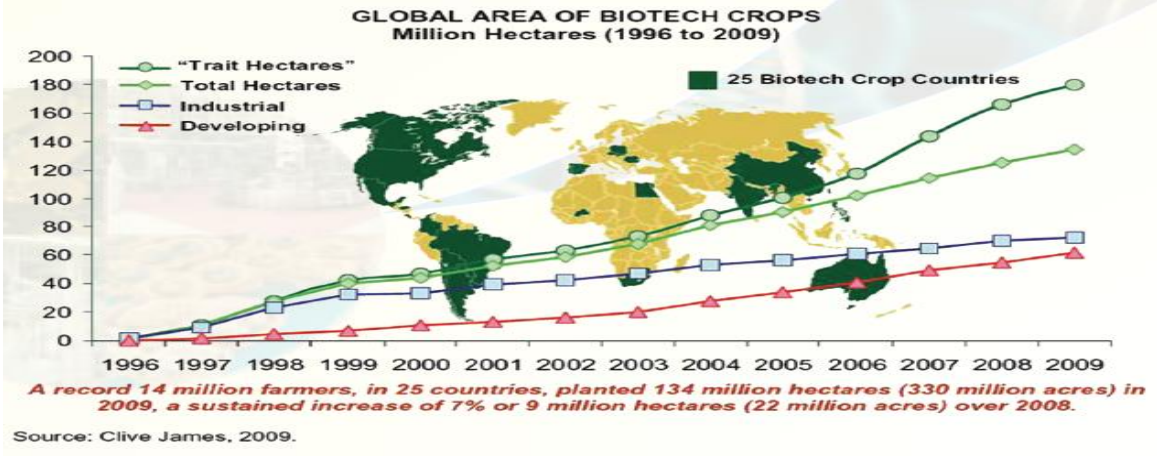
OR

Why does the proteome of cell change when it is infected by virus?

29. GM crops

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 market place (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 commercialisation.



d. Why more and more countries are growing GM crops?
e. What are the advantages of growing GM crops?
f. What are the safety concerns regarding GM crops?

OR

Name the common genetics traits that have been modified in GM crops-

(i) Cotton
(ii) Tomato
(iii) Corn
(iv) Soybean

SECTION E

30. a. What is the significance of RM system in bacteria?
b. Why do we use type II RE in RDT experiments?
c. Mention the rules of nomenclature with the example of *Eco*RI restriction endonuclease.
[2+1+2=5]

OR

a. Name the two enzymes that are commonly used in an RDT experiment along with their functions.
b. How do the fragments generated by *Alu*I differ from the fragments generated by *Eco*RI? Which enzyme will you prefer in RDT experiment and why? [2+3=5]

31. a. Name any four enzymes that function with charge relay system.[2+3=5]

b. How does the charge relay system function in proteolytic enzymes? Explain briefly.

OR

Why is sickle cell anaemia considered a molecular disease? Discuss the process of peptide mapping with an example [5]

32. Compare different types of microbial cell culture with characteristic graph showing [S], [QS] and [X]. Which of these culture types is-

a. continuously fed with fresh medium but not removed

b. used for production of microbial biomass and metabolites [3+2]

OR

a. Label A, B, C and D in the diagram of the bioreactor?

b. Identify the type of bioreactor in the diagram.

c. How are the industrial bioreactors sterilized?

d. Why the pH of the medium is constantly monitored?

e. What is the function of the following parts of a bioreactor?

1. Sparger
2. Impeller

[1+1+1+1+1]

33. What is Embryonic Stem (ES) cell? Where are these cells derived from? How are they maintained in culture? Mention any 2 properties of ES cells.

OR

How is secondary cell culture of animal cells done? Draw a flow chart to show the steps of secondary cell culture. How are cell lines established?

B

Chapterwise- 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	Molecular evolution

	(1962)	
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

B

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-Acetlycholine 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

Chapter 6: ANIMAL CELL CULTURE AND APPLICATIONS

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


तत् त्वं पूषन् अपावृणु
केन्द्रीय विद्यालय संगठन
Kendriya Vidyalaya Sangathan
18, Institutional Area
Shaheed Jeet Singh Marg
New Delhi – 110016 (India)
Phone : +91-11-26858570