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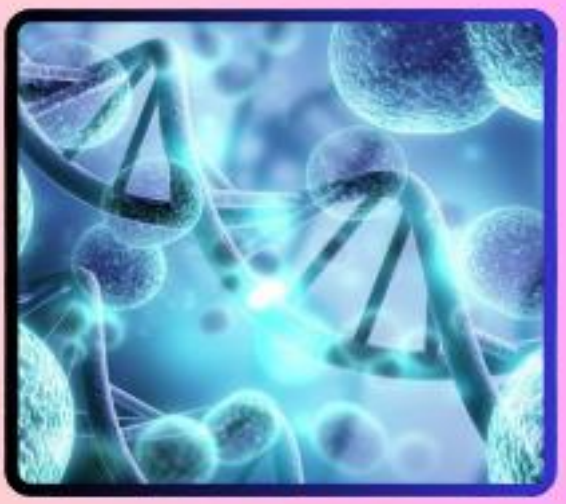
2023-24
स्नातकोत्तर शिक्षक
(जीवविज्ञान एवं जैव-प्रौद्योगिकी)

आज़ादी का अमृत महोत्सव

In-Service Course (ISC)
2023-24
For Post Graduate Teacher (Biology & Bio-Technology)



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VOLUME IV

MINIMUM LEARNING MATERIAL

CLASS XII- BIOTECHNOLOGY

INDEX

CHAPTER 1: RECOMBINANT DNA TECHNOLOGY

GIST OF THE CHAPTER

- Formation of recombinant DNA and its introduction into host cells may be utilized for gene cloning or expression of genes into desired proteins.
- The **basic steps** of recombinant technology are -

1. Isolation of desired gene/ insert gene from the source DNA
2. Formation of a recombinant DNA molecule by ligating the insert gene and dis-armed vector.
3. Transfer of recombinant DNA into suitable host cell that is known as transformation.
4. Selection of true recombinants.

- The first recombinant DNA molecule was established by **Herbert Boyer, Annie Chang and Stanley Cohen in 1973.**

Tools of recombinant DNA technology-

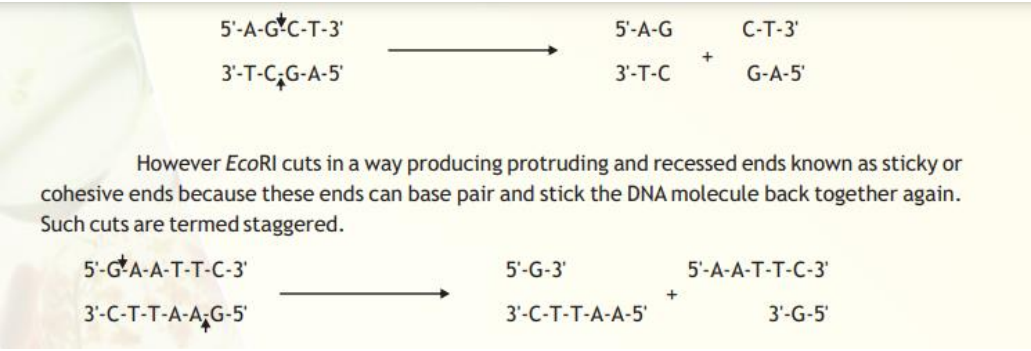
A. Enzymes:

1.Restriction endonuclease enzyme -The source of this enzyme is bacteria. This enzyme shows two different activities.

One is **Restriction activity** which restricts the entry of bacteriophage into bacteria by digesting phage genome.

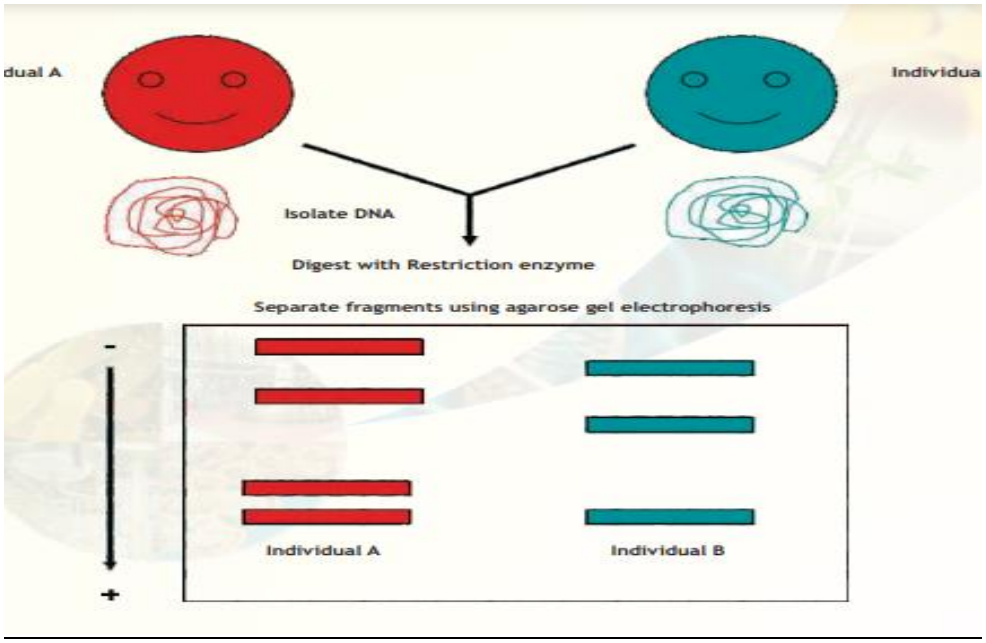
Second is **Modification activity** which results into methylation of bacterial genome, which prevents its own DNA from being digested by the enzyme.

- Only **type II** restriction endonuclease are used in recombinant DNA technology as they introduce cut within the recognition site.
- **Recognition sites** or the **restriction sites** are **palindromic** DNA sequences on a DNA, within which restriction enzymes make cut. Restriction enzymes are also known as **molecular Scissors** and first discovered by **W. Arber, Smith and Nathans** in 1978 for which they got Nobel prize.
- R.E.s make two different types of cuts in DNA molecule- **blunt / flush / symmetrical** cuts which are not considered suitable for RDT experiments.eg. *Alu I* and *Hae III* are the blunt end cutters.



The second type of cut is **sticky or cohesive or staggered** cuts which produces **overhangs** in the DNA molecule so easy to ligate and **preferred for the RDT experiments.**

Restriction fragment length polymorphism



- restriction fragment length polymorphism is a technique to compare the DNA sequences of different individuals.
- The length of restrictions fragments in different individuals vary which is the principal of restriction fragment length polymorphism. This technique is used for DNA fingerprinting.

2. **DNA ligase** - which make phosphodiester bonds between adjacent nucleotide and thus join two DNA fragments. The source of DNA ligase is bacteriophage T4.

3. **Alkaline phosphatase-** It is used to avoid self-ligation of dis- armed vectors by removing the 5' phosphate group from the vectors.

B. Vector:

- vector is a vehicle for cloning.
- An ideal vector must have:
    1. an origin of replication.
    2. Selectable marker genes ex. antibiotic resistance gene or the lac z gene.
    3. It must have recognition sites (minimum 1 and if more than one recognition sites are present in a vector these combine known as multiple cloning site, MCS or polylinker site.
    4. It should be small in size(>10Kb)

- **Plasmid based vectors -** plasmids are extra chromosomal, self-replicating, circular double standard DNA molecules. These are found naturally in many bacteria and many yeast. The first one plasmid-based vector is **pBR 322** which has an origin of application two selectable marker genes (first is ampicillin resistance gene and second is tetracycline resistance gene). The Bam H1 recognition site is present within the tetracycline resistance.
- The second plasmid is **pUC 18** which has origin of replication, ampicillin resistance gene and MCS within the lac Z gene (codes for beta galactose enzyme which converts a component of culture media that is x gal into a blue colour product.
- Yeast episomal plasmid (**YEP**) which is a **shuttle vector** as it can be used for eukaryotic as well as a prokaryotic cell. YEP, has Ori and the Autonomous Replicating Sequence (ARS) respectively for self-replication in prokaryotic and eukaryotic cell. Ampicillin resistant gene and Leucin 2gene are

used as a selectable marker for prokaryotic and eukaryotic cells as well. It forms the essential amino acid Leucin so that transformed cells will not require Leucin from the culture media.

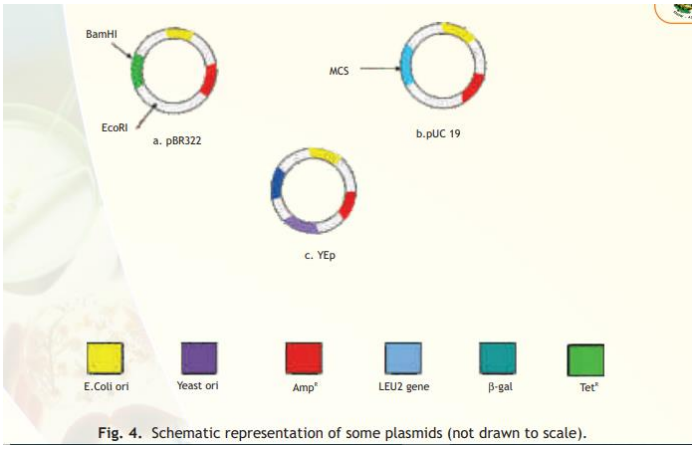


Fig. 4. Schematic representation of some plasmids (not drawn to scale).

- **<u>Bacteriophage based vectors</u>** are of two types- Lambda phase and M-13 Phage.

lambda phase bacteriophage- it's genome is 48 Kbp long and has 12 bases on each end which are unpaired and complementary so called **Cos sites or the cohesive sites.** It has double standard and linear DNA and for making it as a vector, the genes responsible for lytic infection are replaced by insert gene. This vector can accommodate an insert of 23 Kbp.

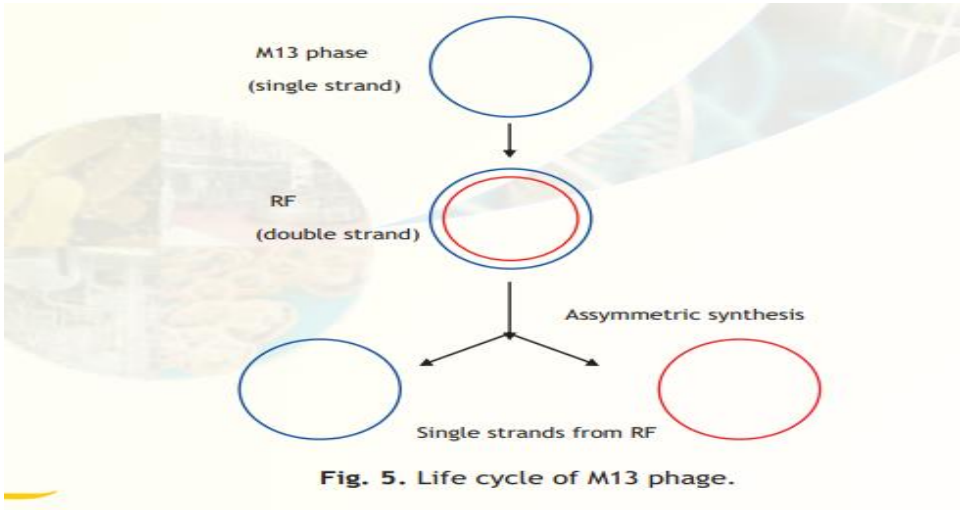


Fig. 5. Life cycle of M13 phage.

M-13 genome is only 6.4 Kbp long single standard and circular DNA. Vector based on this has more advantages than the lambda phage as –

- Its size is less than 10 KB.
- It does not show lytic pathway and simply diffuse out of the host cell so the whole cells will not be destroyed.
- The insert gene can be obtained in single standard form as well as double stranded form. This is the unique property of M13 that is why it is used in DNA sequencing and site directed mutagenesis techniques.

- Cosmid: It has the sequences from the lambda phase and plasmid .it can accommodate an insert of 45 K bp.
- YEP: Yeast artificial chromosome- these were used in human genome project. the accommodation capacity is 250-1000Kbp.
- BAC: bacterial artificial chromosome- it can accommodate inserts up to 50-500 kbp.
- Animal viral vectors - SV40 virus, papillomavirus and retro viruses.

- Plant viral vectors – cauliflower, tobacco mosaic and Gemini virus.

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

C. HOST cells- mostly for prokaryotic gene, *E.Coli* cells are used and for eukaryotic gene, yeast cells are preferred. As both of these kinds of cells are very simple in organization, the genetic makeup has been extensively studied, these are easy to handle and grow and they can accept a range of vectors.

Making of recombinant DNA

Dis-armed vector and the insert gene are ligated together with the help of DNA ligase enzyme and later on introduce it into the host cells using any of the method like transformation, transfection, electroporation, microinjection, biolistic etc.

Screening for recombinants:

After transformation,3 different kind of cells are observed. Non-transformed cells, transformed non recombinant cells and recombinant cells. So, we need to screen third kind of cells. On the basis of the selectable marker present in the vector, the screening method would be adopted. There are different two type of screening –

a.) **the Replica plating** based on the antibiotic resistant gene and

b.) **the visual screening** that is based on the lac Z gene

- **PCR reaction** (polymerase chain reaction)
- it was invented by Karry Mullis in 1985 and used for amplification of a specific fragment of DNA molecule.
- Three major steps:

  1.**Denaturation of DNA molecule**: DNA molecule is heated up to high temperature. Both the strands of DNA get separated.

  2. **Annealing**: Promoters are allowed to anneal with the single stranded template at 50-60'C.

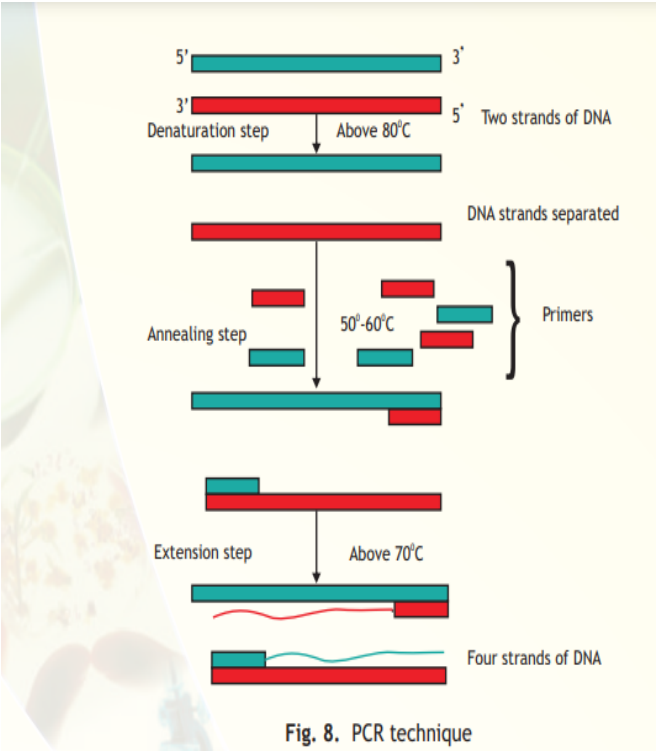


Fig. 8. PCR technique

3. **Extension:** Taq DNA polymerase is used to extend the primers and making the DNA molecule at 70-74'C. The normal DNA polymerase cannot polymerize so a DNA polymerase isolated from *thermophilus Aquaticus*.

PCR based diagnosis is faster safer and more specific as it does not use live pathogens.

DNA sequencing / Dideoxy nucleotide chain termination method

It was first developed by Frederick Sanger and Andrew Coulson.

Principal: An unusual nucleotide, di deoxy nucleotide, lacks 3'-OH group because of this, it can't extend DNA molecule by making phosphodiester bond and terminate the chain.

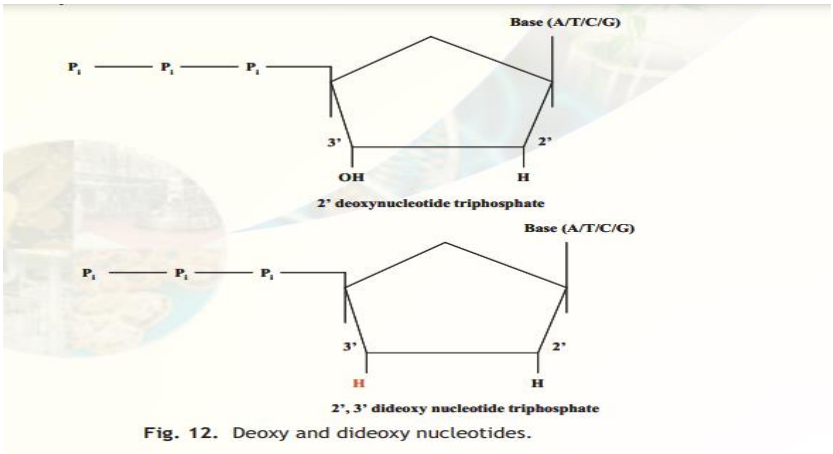
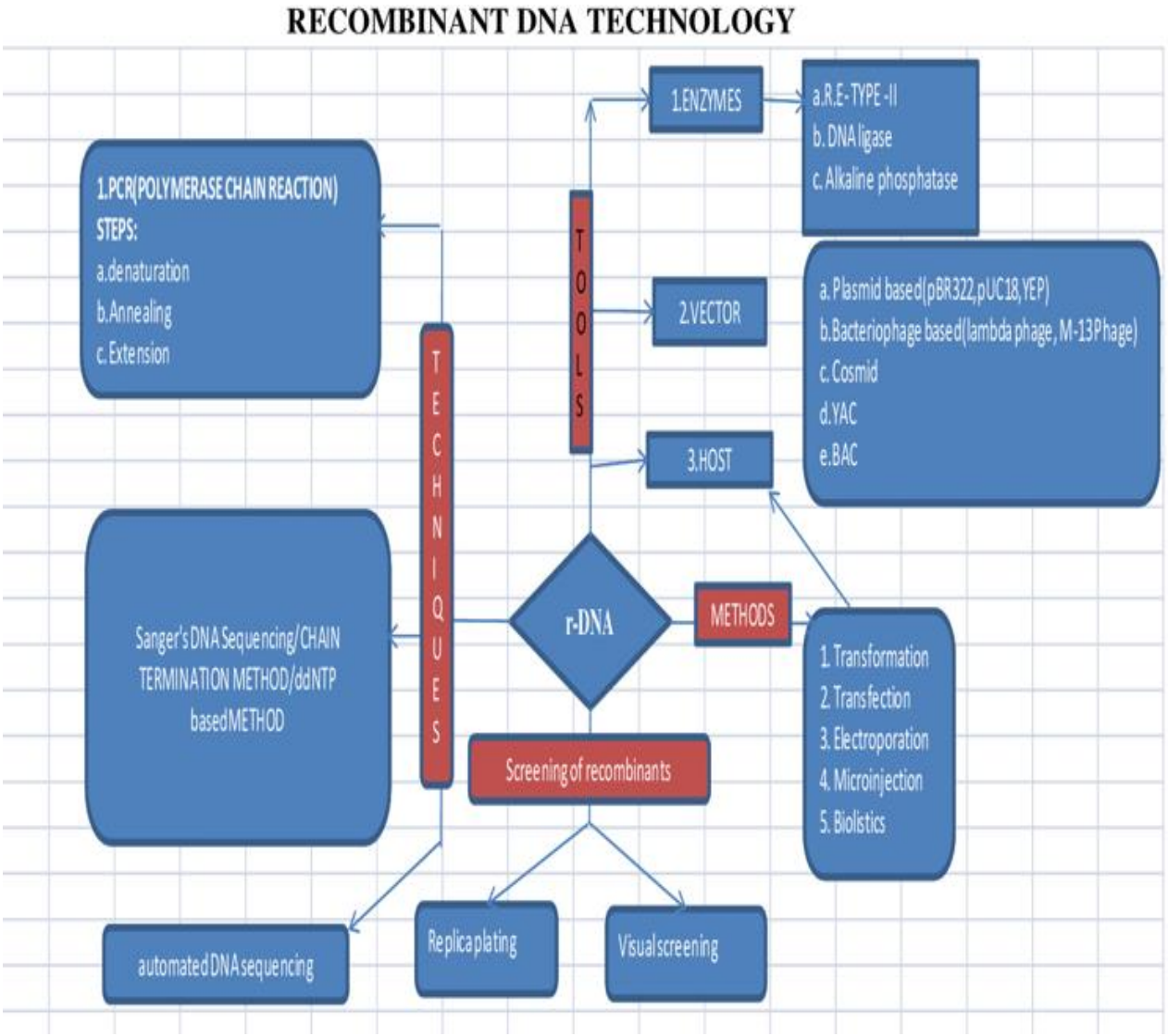


Fig. 12. Deoxy and dideoxy nucleotides.

Advance method of DNA sequencing has been developed and the four different ddNTPs labelled with different fluorochrome molecules so the whole reaction can be completed in a single test tube and give appropriate results.

MIND MAP



MULTIPLE CHOICE QUESTIONS

1. --------- cells were used to produce hepatitis B vaccine-

   a. Yeast b. plant c. insect d. human

2. The first recombinant DNA molecule was established by the combined efforts of –

   a. Paul berg, Herbert Boyer, Annie Chang and Stanley Cohen

   b. Arber, Smith and Nathans

   c. Paul Berg, Smith and Nathans

   d. Nathans, Paul Berg, Stanley Cohen

3. Choose the flush end cutter enzymes-

   a. *EcoRI*

   b. *AluI*

   c. *BamHI*

d. *PstI*

4. Bacteria protects its own DNA from being digested by restriction enzymes using-

a. Methylation b. Modification c. Restriction d. Both a and b

5. Basic principle of RFLP is-

a. DNA isolated from an individual has a unique sequence

b. Restriction fragments vary in length in different individuals

c. Restriction sites would vary in different individuals

d. All above

6. Individuals except ------- vary in their RFLP patterns –

a. mother and son b. siblings c. identical twins d. father and son

7. An ideal vector should have-

a. small size b. has limited number of cloning sites

c. not be self-replicative d. avoid a selectable marker

8. Which of the following vector is suitable for an insert of 1000 kilobytes size-

a. YAC b. BAC c. Cosmid d. plasmid

9. In order to make competent host cells –

a. host cells are treated with cold calcium chloride solution

b. host cells are heated up to 250 degrees Celsius

c. host cells are directly introduced with vector molecule

d. host cells are treated with DEAE Dextran

10. Name the enzyme involved in visual screening of recombinants-

a. beta galactosidase

b. beta galactose

c. X-Gal

d. Restriction endonuclease

11. Identify the correct sequence of reactions in a PCR-

a. Denaturation, annealing, extension

b. annealing, denaturation, extension

c. extension, annealing, denaturation

d. annealing, extension, denaturation

12. PCR stands for

a. primer chain reaction

b. polymerase crime reaction

c. polymerase chain reactant

d. polymerase chain reaction

13.Five molecules of DNA undergone PCR. How many DNA molecule will be produced after five cycles-

a. 80 b.160 c.320 d.260

14.PCR based diagnosis is-

a. slower, less safe and less specific

b. faster, safer and more specific

c. time taking and laborious.

d. faster, less safe and more specific

15. In what way ddNTP is different from dNTP-

a. It lacks 2'OH

b. It lacks 3' OH

c. It has 2 molecules of -OH groups at 3'

d. It has 3 molecules of phosphate groups at 5'

16. for DNA sequencing, the chain termination method was invented by –

a. Fred. Sanger and Andrew Coulson

b. Paul berg and Nathans

c. Gilbert and Allan Maxam

d. Karry Mullis

17. Thermocycler was invented by –

a. Karry Mullis

b. Gilbert

c. Sanger

d. Nathans

18. In PCR, extension of primers is carried out by-

a. at 70- 74 degree Celsius by Taq DNA polymerase

b. at 70- 74 degree Celsius by DNA polymerase

c. at 37 - 40 degree Celsius by DNA polymerase

d. at 37 - 40 degree Celsius by Taq DNA polymerase

19. The first cloning experiment involving mammalian cells, used a vector based on

a. Adenovirus b. Papillomavirus c.SV40 virus d. Retrovirus

20. Cosmid can be used to clone DNA fragment up to the size of-

a. 45 Kbps b.50 Kbps c.100 Kbps d.145 kbps

CHAPTER 2: PROTEIN STRUCTURE AND ENGINEERING

GIST OF THE CHAPTER

- **Proteins among the other biomolecules have the maximum diversity in functions** because they are made up of 20 different amino acids and the unique sequence and combinations of these amino acids make the structure of a protein unique and because of that proteins attain millions of unique 3-dimensional structures and therefore functions.
- Different kind of proteins are present in our body- Myosin and actin protein which are present in muscles, collagen protein which make collagen fibres and are present almost in all the different kind of cells, haemoglobin-present in RBC.
- Protein related abnormalities-
  1. **Thalassemia** it is because of absence of a chain of protein. In beta thalassemia the beta chain of haemoglobin is totally absent.
  2. **Sickle cell anaemia** an amino acid substitution at 6th position in beta chain of haemoglobin. Glutamic acid is substituted by valine amino acid.
  3. **SCID (Severe combined immunodeficiency)** adenosine de-aminase enzyme is absent in the patient.
  4. **Mad cow disease** in cattle caused by the Rogue proteins / prions or infectious distorted proteins.
- **3D shape of proteins**: many of scientists contributed in elucidation of structures of proteins and names of some are- **Pehr Edman** who developed protein sequencing method after noble prize winner scientist **Frederick Sanger** who first developed protein sequencing method. the first sequenced protein was insulin. **Linus Pauling** derived the secondary structure of proteins (α- helix and beta pleated sheet). **Ramchandran** developed the Ramchandran's plot which informs the kind of secondary structure present in a protein. **Max Perutz** elucidated the structure of **haemoglobin**. **John Kendrew** explained the structure of myoglobin.
- **Eli Lilli** was the first biotech company to develop recombinant human insulin.
- **Different kind of bonds** present in the proteins are –
  1. **Covalent bonds** ex: Peptide bond and Disulphide bond.
  2. **Non covalent bonds** which mainly contribute tertiary and quaternary structure. Ex. Ionic bond /Salt bridge, hydrogen bonds, Vander wall bonds and the hydrophobic interactions. **The hydrophobic interactions are the most important kind of forces in driving proteins to fold into compact global structure in water**.
- **Structure function relationship in proteins:** A protein's function is regulated by its structure. If there is a change in the structure automatically the function will be affected example is Chymotrypsin which is a proteolytic (also called protease) enzyme and synthesized by pancreas in its inactivated form that is chymotrypsinogen which is a zymogen. **Zymogens are the inactivated form of proteolytic enzymes.**

  Later on, this is released into duodenum which is the site of action of this enzyme.

  In situ activation and **molecular alteration** of chymotrypsin takes place in duodenum where another proteolytic enzyme trypsin makes a proteolytic cut on chymotrypsinogen which leads the conformation changes and expose the substrate binding pocket and convert it into activated chymotrypsin enzyme.
- Chymotrypsin **has 245 amino acids** interrupted into three peptide chains a, b and c.

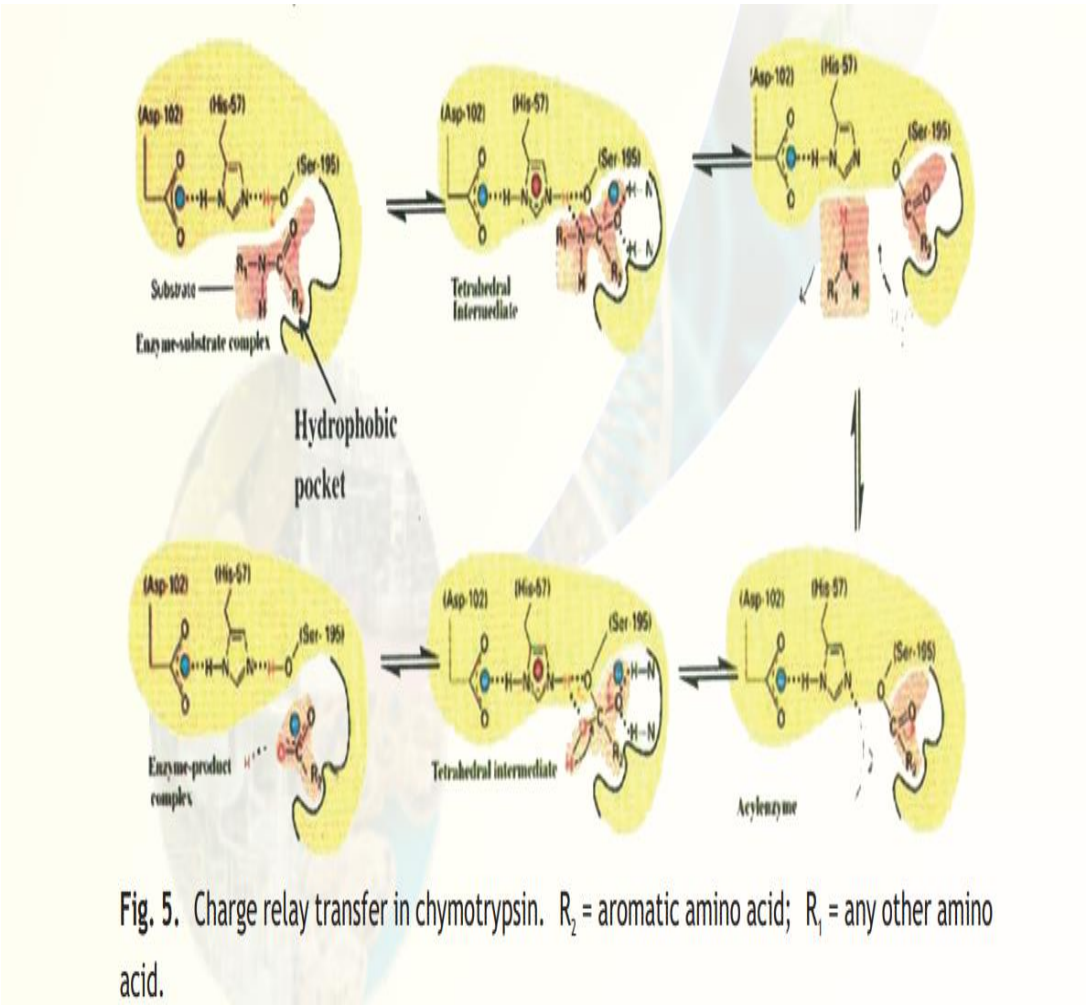


Fig. 5. Charge relay transfer in chymotrypsin. R_2 = aromatic amino acid; R_1 = any other amino acid.

- In the active chymotrypsin a **Charge relay system** operates which is the basis of its catalytic activity. The unique constellation of three amino acids-Aspartate 102, histidine57 and serine195 make the active site of the enzyme which is easily accessible for the substrate (protein). Negatively charged Aspartate borrow H^+ from histone 57, which in turn borrow H^+ from Serine195. In this way Serine195 becomes acidic as it attains an oxyanion and undergoes hydrophilic reaction with protein substrate by making a cut on peptide bond after aromatic amino acids.
- Other proteolytic enzymes examples are- subtilisin, thrombin, brain enzyme acetyl choline esterase.
- **Competitive inhibitors** –selectively react with an acidic serine and inhibit catalytic activity of enzyme.eg:1.**Nerve gas**, used in first world war (has volatile Serine alkylating compounds which inactivate the brain enzyme acetyl choline esterase), **organophosphate** like **malathion, parathion used as mosquito repellents**.
- **Sickle cell anaemia** is also known as a molecular disease because **substitution of amino acid** takes place in the haemoglobin protein and **protein is a biomolecule** that is why it is also known as a **molecular disease**.
- first of all, **Linus Pauling** predicted that the sickle cell haemoglobin molecule is having lesser electrophoretic mobility in comparison to the normal haemoglobin.

- later on, it was confirmed by **V.M. Ingram** scientist in 1957 by using a technique that is **Protein finger printing or Peptide mapping**.

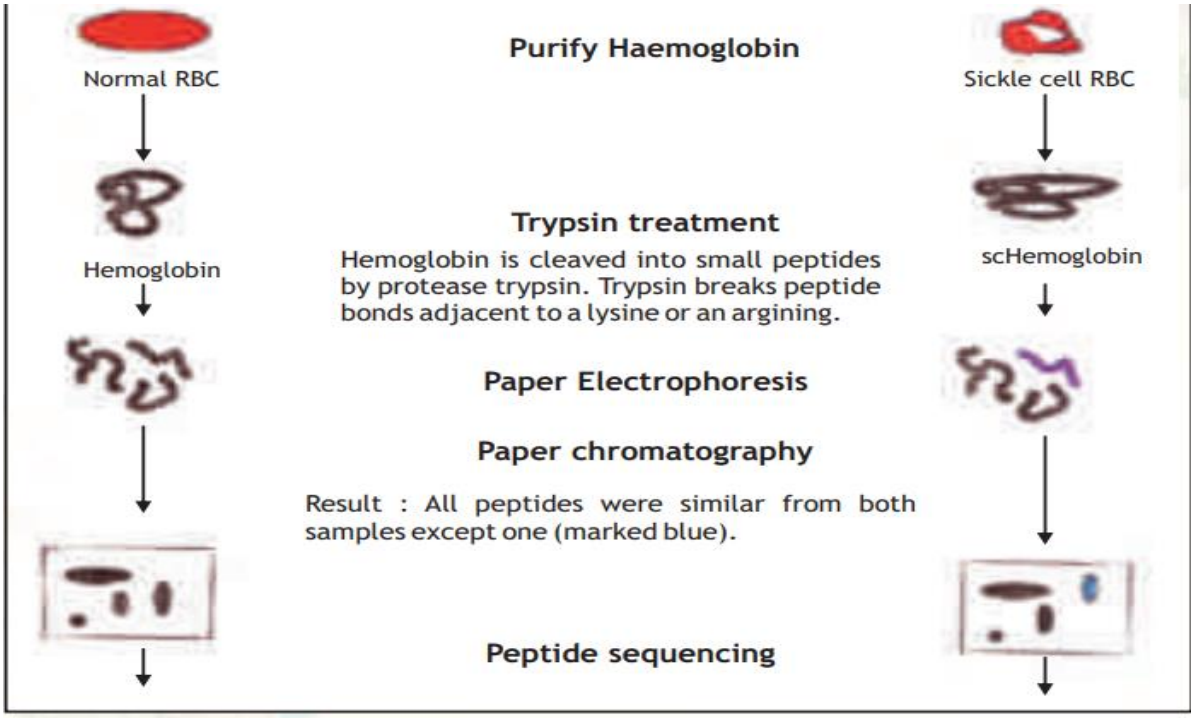


Fig. 6. Protein fingerprinting

- **Steps of PFP**: 1. the normal haemoglobin and sickle cell haemoglobin separated from the cells. 2. Allowed for trypsin cut. 3. Protein fragments separated using paper electrophoresis .4. On the right angle of it, allowed for paper chromatography. 5.Ninhydrin spray was done on both chromatography sheets. 6.OrangeYellow spots are appeared .7. Observed one spot differently located in Sc map.8. Peptide in different spot was sent for sequencing. 9. Concluded the glutamic acid is substituted by valine amino acid in haemoglobin beta chain at 6th position.
- **2D electrophoresis** (two-dimensional electrophoresis)- developed by O'Farrell. 2 D-gel electrophoresis is a combination of two different techniques. One is IEF, isoelectric focusing and the another one is SDS PAGE.
- **Isoelectric focusing** technique separates the protein fragments on the basis of their pI value or charge. pI is equal to that pH where a protein's net charge becomes zero. So the peptide fragments of the proteins will not further run in the pH gradient setup with the help of ampholytes in a test tube.
- **SDS PAGE (Sodium Dodecyl Sulphate Poly Acrylamide Gel Electrophoresis)** separates the proteins on the basis of **their size or molecular weight**.
- **2D PAGE is better than the normal electrophoresis** as its separates the protein fragments on the basis of different two parameters- pI /charge and molecular weight, hence a better resolution we find in 2-dimensional gel electrophoresis.
- **Characterization of proteins** -for it we use different techniques-
  1. SDS PAGE
  2. Protein fingerprint
  3. Gel electrophoresis
  4. Protein sequencing and

5. **Mass spectrometry (MS**)-is the **modern** technique for characterizing the proteins. Peptide mass, amino acid sequence, structure of a protein, type and location of amino acid modification etc can be known. This is a very sensitive and efficient method which is the major attraction of this technique as a very little picomoles (10^{-12}) of a protein can also be analysed.

The principle of this technique is proteins are first converted into respective ions and mass by charge ratio is analysed for each of the ion for further detection. The steps of this equipment are ionization, acceleration, deflection, detection and amplifier etc.

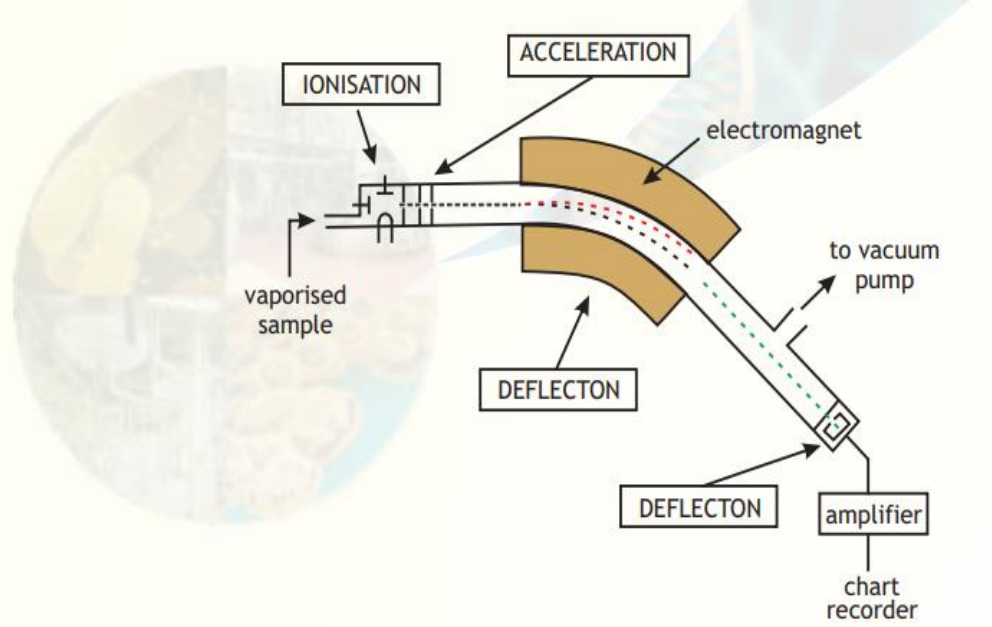


Fig. 10. An outline of a mass spectrometer.

MALDI MS (Matrix assisted laser desorption ionization) -The proteins are first converted into respective ions. Sample protein from a condensed phase is transferred into a gas phase with the help of a solid matrix and this is achieved by directing the laser beam on to a sample suspended or dissolved in a matrix. The ions are later on detected for their mass by charge ratio and at last provide the required information in the form of graph.

- **Protein based products** which are commercially available or being commercialized are under the category of-

1.

S.No.	Protein based product category	Commercialized product
1.	blood products and vaccines	• factor VIII (treats hemophilia A) • factor IX (treats hemophilia B) • hepatitis B vaccine
2.	Therapeutic antibodies and enzymes	• Tissue Plasminogen Activator (tpa, digests block in arteries following myocardial infractions) • Monoclonal antibody (MAb, OKT-3 used to prevent rejection of transplanted graph.
3.	Growth factors	• Humulin protein • Platelet derived growth factor (PDGF)to cure skin ulcer
4.	Regulatory factor	• Interferon(INF) α is used for hepatitis C, • INF β for multiple sclerosis and

		• INFϒ for chronic granulomatous
5.	Analytical application	• Hexokinase for quantitative estimation of glucose in serum, • Uricase enzyme for uric acid level estimation in serum, • Horse radish peroxidase and alkaline phosphatase in ELISA
6.	Industrial enzymes	• Alcalase enzymes in soap industry, • Papain in beverage industry, • Glucose isomerase in confectionery industry and • Chymosin in cheese industry
7.	Functional non catalytic proteins	which have properties such as emulsification, gelation, water binding, whipping and foaming etc. ,eg : kappa casein*
	Nutraceutical proteins	provide us nutrition as well as cure against disease. Example- whey protein

- *Kappa casein is involved in micelle stabilization of milk proteins and keep the proteins suspended uniformly in milk because it behaves like a lipid molecule (2/3rd of this protein is hydrophobic)
- **<u>In ancient times whey was used for many kind of therapeutics</u>** as-
  1. These proteins result in elevation of a tri peptide (glutathione, gamma glutamyl cysteinyl glycine) in cells.
  2. This tripeptide is a reducing compound and detoxifying agent so give protection to cellular components from oxygen intermediate and free radicals thus acts as an antioxidant.
  3. The biological value (B.V.) of whey protein is maximum
  4. The PER (protein efficiency ratio) is highest.
  5. It is a good source of branched amino acids.

  functional non catalytic protein:

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.

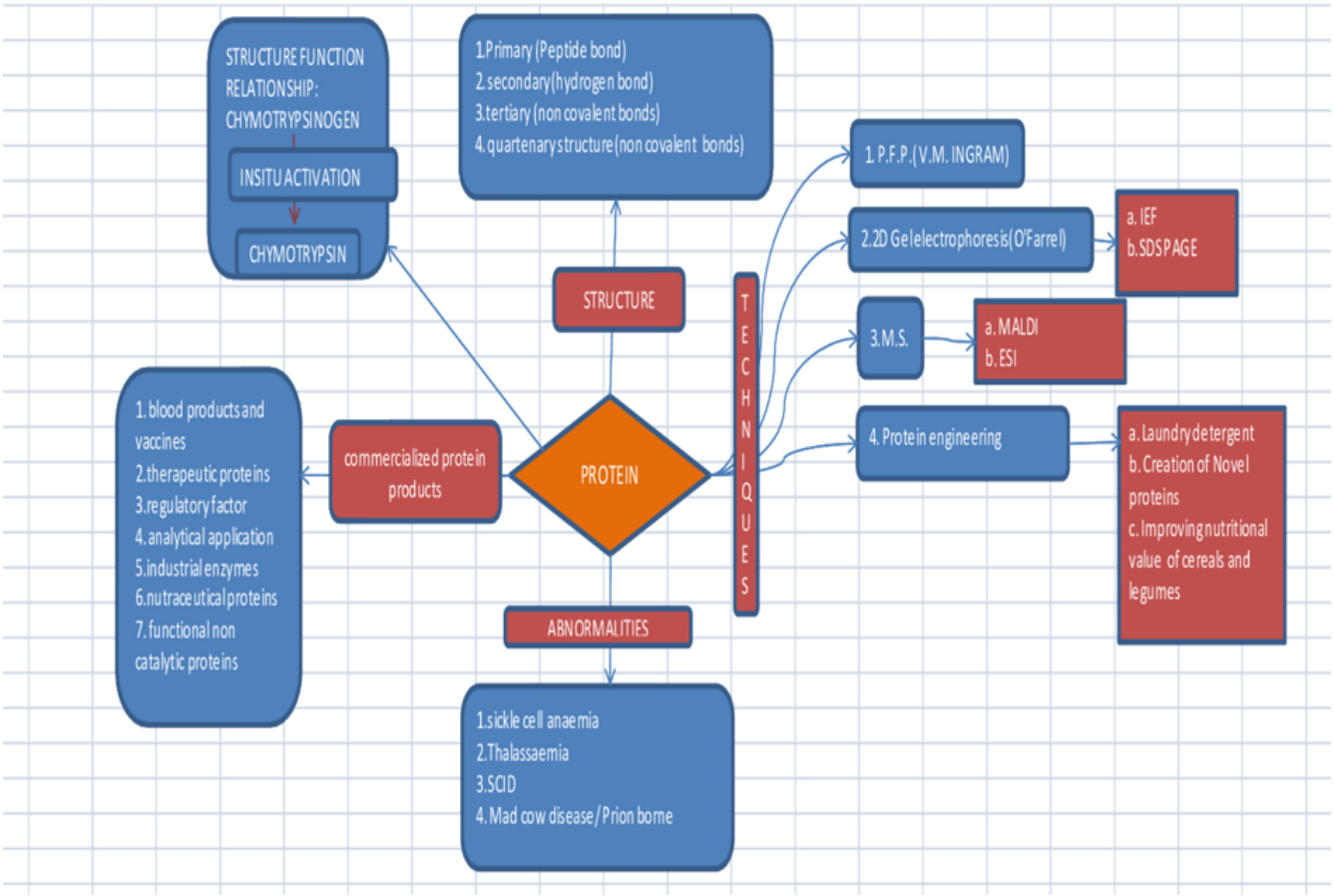
- **Curd is considered as a probiotic** because it is a good source of beneficial bacteria which can colonise the intestinal tract.
- **Protein engineering** is designing of proteins in such a way that we desire. It is achieved for the improvement of one or the other quality of a protein like –

- to increase the stability in hash conditions like elevated temperature
- tolerance for organic solvents, reactive chemicals,
- increased stability in extreme pH,
- increased catalytic potential.

- In order to engineer a protein first the cause of inactivation of protein should be analysed as we know that the stability of a protein is a consequence of its unique structure and sequence of amino acids.
- Examples of protein engineering –
  1. **Improving laundry detergent Subtilisin:** subtilisin is a proteolytic enzyme which is isolated from a bacteria *Bacillus subtilis.* It is used in detergent industry for removing the stains of protein and the detergent having this enzyme have catchy slogans like "stain cutter or biological active enzymes".

     Subtilisin in detergents gets inactivated when it is used with bleach as bleach oxidizes Methionine residue at 222 positions so this Methionine222 was substituted with Alanine, which resulted in retained activity of subtilisin in the presence of bleach.
  2. **Creation of novel proteins**: novel vaccines have been created using recombinant DNA technology and one of such examples is hepatitis b virus vaccine.
  3. **Improving nutritional value of cereals and legumes:** Cereals and legumes are considered unsuitable for a balanced diet because of the deficiency of certain essential amino acids. so grains and seeds of legumes can be supplemented with the amino acids which are usually deficient or absent in these essential amino acids.
- **Essential amino acids** which cannot be synthesize in our cells by the different metabolic reactions and need to obtain from our food.
- Some of the **BCAA** (**branched chain amino acids) are- Leucine, Isoleucine, valine, lysine, tryptophan.**
- These branched chain amino acids are essential for the biosynthesis of muscle protein so **athletes are advised to consume BCAA rich food before and after exercise to maintain their existing body mass** as these BCAAs help in increasing the bio availability of high complex carbohydrates intake and absorbed by muscle cells for the muscle building activity. During exercise BCAA released from the skeletal muscles, carbon skeleton is used as a fuel and nitrogen is used to make Alanine which later on in liver turned into glucose for energy.
- **Biological value (BV)** of a protein measures the amount of protein nitrogen that is retained by the body from a given amount of protein nitrogen that has been consumed.
- **Protein efficiency ratio (PER**) is a measure of growth expressed in terms of weight gain of an adult by consuming 1 gram of food protein.
- The PER value of different proteins is arranged in following **decreasing order** –
  Whey>milk> casein> Soya> rice > wheat

MIND MAP



MULTIPLE CHOICE QUESTIONS

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

(a) Acceleration, deflection, detection, ionization

(b) Ionization, acceleration, deflection, detection

(c) Acceleration, ionization, deflection, detection

(d) Acceleration, deflection, ionization, 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 anemia occurs:

a. Sixth position in alpha chain of hemoglobin

b. Sixth position in beta chain of hemoglobin

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 pI.
   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 pI 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 anemia 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. INF μ

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 noncovalent bond in driving proteins to fold into compact structures in water is-

a. hydrogen bonds

b. hydrophobic bonds

c. Vander wall 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 biomolecule displays maximum diversity in function-

a. Protein

b. Lipids

c. Carbohydrates

d. Nucleic acids

ANSWERS:

1	A	8	A	15	B	22	D	29	D
2	A	9	A	16	A	23	B	30	B
3	B	10	A	17	A	24	C	31	C
4	D	11	A	18	A	25	B	32	A
5	D	12	D	19	C	26	B	33	D
6	C	13	B	20	A	27	A	34	B
7	A	14	B	21	B	28	C	35	C
36	D	37	D	38	D	39	D	40	A

PREPARED BY: MRS PUJA KULTHIA

PGT BIOTECHNOLOGY, K.V NO1 PATIALA, CHANDIGARH

CHAPTER 3: GENOMICS & BIOINFORMATICS

GIST OF THE CHAPTER

1. Genomics (Genome + omics)

In 1987, the term "Genomics" was coined by Thomas H. Roderick. Genomics means sequencing and mapping the genome to analyse its structure and organisation.
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.

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.

3. Proteomics (Proteome + omics)

Proteomics is the study of gene products encoded by a genome: -

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
Proteome	The complete set of proteins encoded by the genome
Proteomics	Determination of the proteome
Bioinformatics	Management and interpretation of data generated by genomics

4. 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.**

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. Gene Prediction and Counting

a) Gene Prediction

- After a genome sequence has been obtained and checked for accuracy, the next task is to find all the protein coding genes (Gene Prediction).
- Locating protein-coding genes is done by inspecting the sequence using a computer software or by eye.
- Genes have several identifying features.

In prokaryotes,

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

In eukaryotes,

(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 software 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.

6. Gene Counting

- Even 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.
- 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

7. Single Nucleotide Polymorphisms (SNPs)

SNPs 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 in 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.

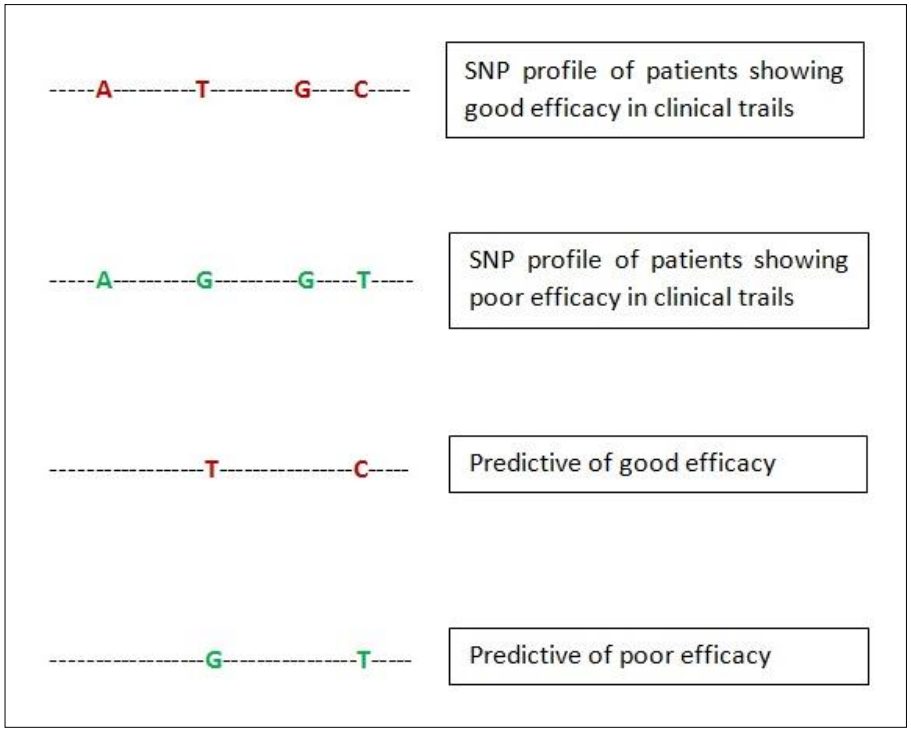
The variations in genetic sequences of different individuals (due to SNPs) are thought to be involved in:

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

8. Pharmacogenomics

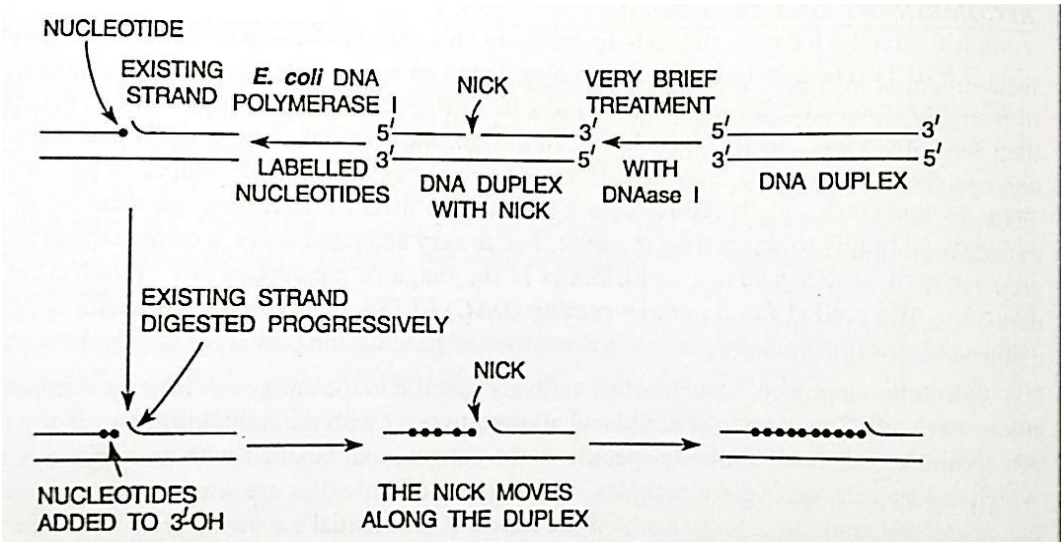
- The field of study that is concerned with the effect of genetic variation on disease susceptibility and drug response is known as pharmacogenomics.
- Pharmacogenomics is expected to develop individualized treatments that will be safer as well as more effective.

- The 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
- **How to predict response to drugs using SNP profile**



9. Nick Translation

- Nick translation is 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.



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

Fig : Nick Translation

10. Fluorescence in situ hybridization (FISH)

- 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.

Process of FISH:

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 42^0C.
d. Allow the fluorescent probe to hybridize with the fixed cells or tissue.
e. Washing to remove unbound probe.
f. Observe under fluorescence microscope to detect the location of the bound probe by detecting the fluorescence associated with probe.

Application of FISH

- In detecting the severity of a disease "chronic myeloid leukaemia" (CML): cancer of white blood cells.
- CML is associated with a reciprocal translocation between Ch9 and Ch22.

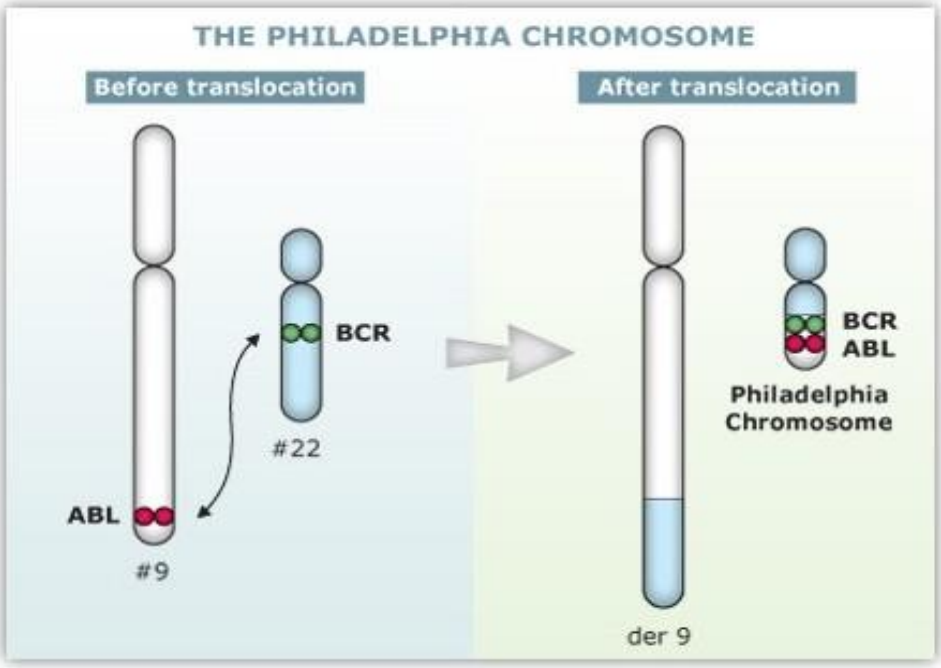


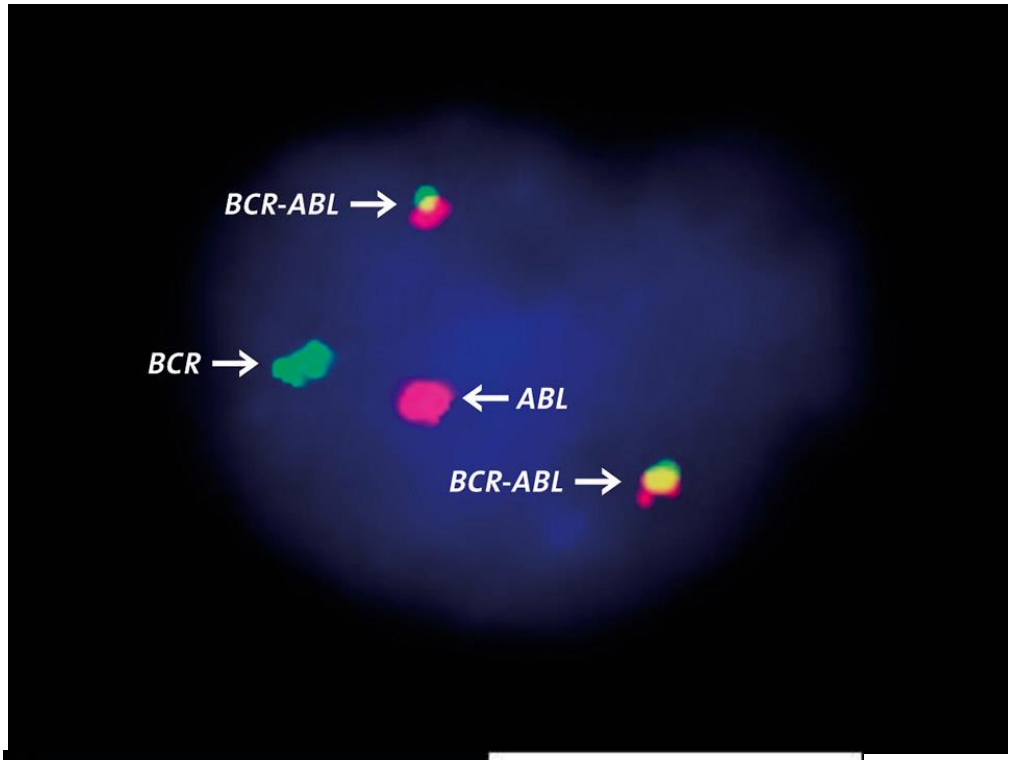
Fig : Reciprocal Translocation between Ch9 and Ch22 to give rise to Philadelphia Chromosome

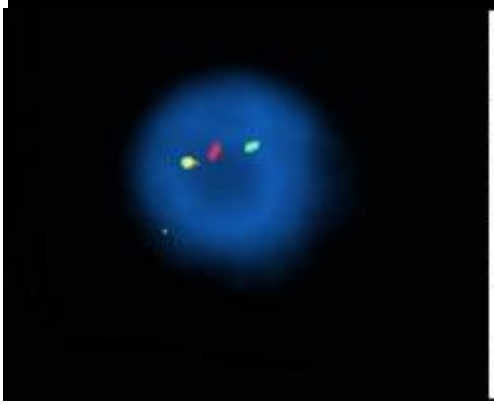
- From the DNA library, it was possible to pick up clones carrying the genes involved in CML (*abl* and *bcr*).
- Using nick translation, it was possible to prepare differently coloured fluorescent probes for both of these genes (*abl* and *bcr*).
- Suppose that the probe for *abl* is tagged with red and the probe for *bcr* is tagged with green fluorescent tag.

Fluorescence in situ hybridization (FISH): Process

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.

11. FISH Images under Fluorescence Microscope



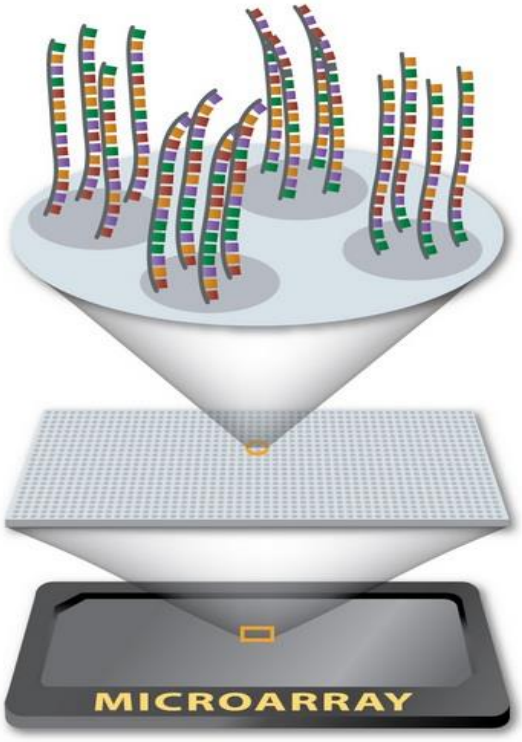


A cell that is BCR-ABL positive by FISH (yellow fusion signal). The yellow signal shows the fusion of the green probe (the BCR portion of chromosome 22) with the red probe (the ABL1 portion of chromosome 9.

Courtesy of
Mary Lowery Nordberg, PhD

12. 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.
- Each spot 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.



Comparative cDNA Hybridization Microarray

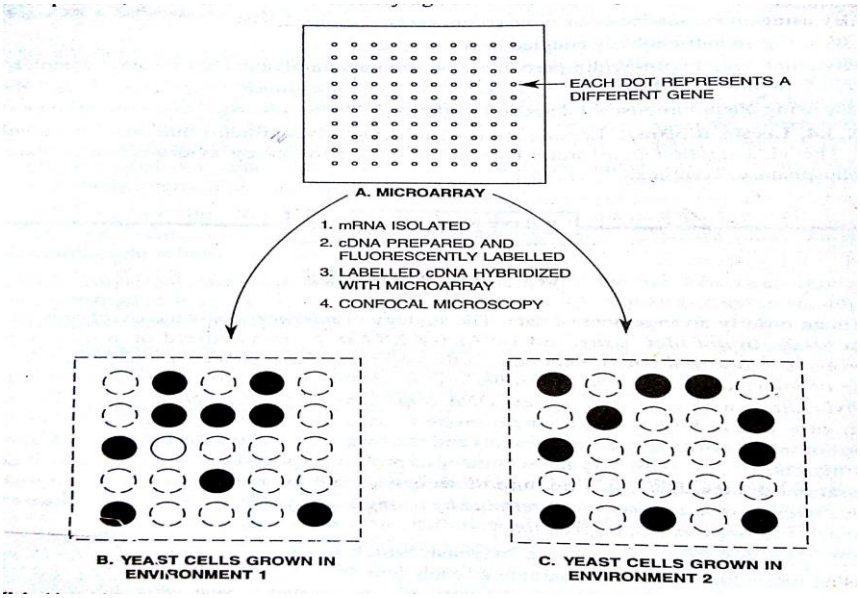


Fig : Use of DNA Microarray to compare gene expression pattern of yeast cells grown in two different environments

13. Use of DNA Microarray

- 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.

14. Proteomics

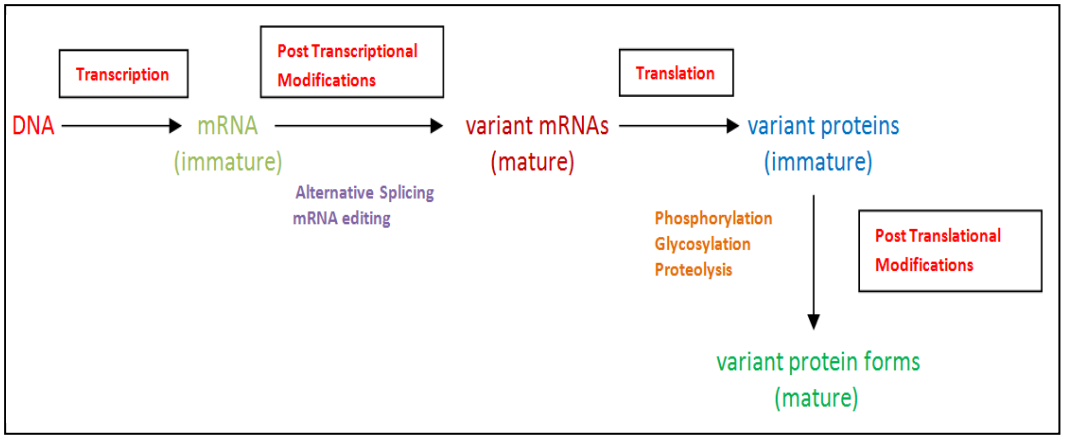
- Proteome: the complete set of proteins expressed during a cell's entire lifetime.
- Proteomics: It is the large-scale study of the proteome of living organisms.
- Study areas:
  1) protein-protein interaction studies
  2) protein function
  3) protein localisation
  4) protein expression

Branches of Proteomics

- **Structural Proteomics:** Mapping out the 3D structure and nature of protein complexes present specifically in a particular cell/organelle.
- **Functional Proteomics:** Use of proteomics techniques to analyze characteristics of molecular protein-networks involved in a living cell.
- **Expression Proteomics:** Quantitative study of protein expression between samples differing by some variable.

No. of Genes v/s No. of Proteins

- Generally, it is assumed that one gene code 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.



15. 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.

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.

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.*

Databases:

Databases are of two types-
(1) Nucleotide sequence Databases (2) Protein databases

Nucleotide Sequence 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

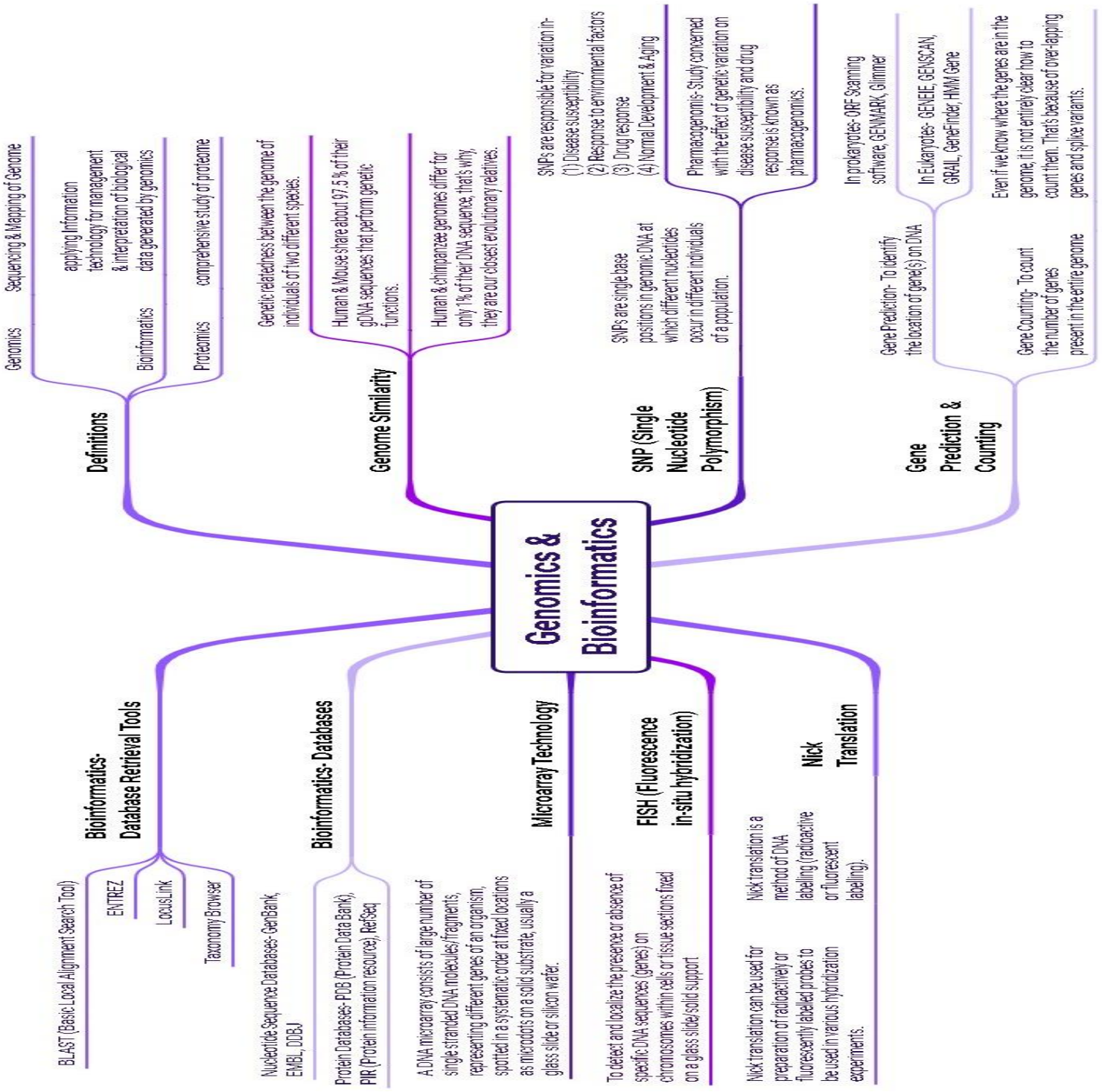
Protein Databases

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

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

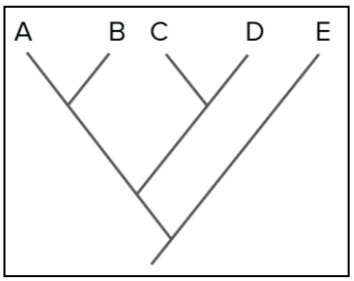
LOCUS LINK (NCBI, USA)	Accessing information on homologous genes, i.e., genes of different species that are similar due to common ancestry
TAXONOMY BROWSER (NCBI, USA)	Taxonomic classification of various species as well as genetic information



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

```
CTGACTAAGTACCGA
CCGACTAGGTACCGA
CCGACTAAGTACCGA
CTGACTAAGTACCTA
CTGACTAGGTACCGA
```

6. How many SNP loci are present in the aligned sequences shown herewith?

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

```
CTGACTAAGTACCGA
CCGACTAGGTACCGA
CCGACTAAGTACCGA
CTGACTAAGTACCTA
```

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

CHAPTER 4 : MICROBIAL CELL CULTURE & ITS APPLICATIONS

GIST OF THE CHAPTER

1. **Microbial Cell Culture-** The act of growing microbial cells under laboratory by providing appropriate nutrients and environment.
2. A microbial culture works as a factory in which the metabolism of a microorganism is exploited to convert raw material into products.
3. **Microbial Culture Media-**

   Microbial culture medium is nothing but a mixture of all those nutrients which are needed by the microbe to be cultured, i.e., carbon source, nitrogen source, energy source, trace elements, growth factors, etc.

Types of Microbial Culture Media		
Synthetic Media	**Semi Synthetic Media**	**Commercial Media**
Simple nutritive medium devised using pure chemicals (like glucose as carbon-source) such that full chemical composition of the medium is known. (suitable for lab-scale culture)	Nutrient medium is a mixture of pure chemical and highly complex components such as yeast extract, beef extract, peptone, etc., so that exact chemical composition of the medium is not known. (can be exercise at lab scale)	Nutrient medium which uses sources of nutrients, which are economical, raw and available readily throughout the year. (suitable for commercial stage production)

4. At commercial scale, in microbial culture media antifoaming agents are added to minimize foam formation.

   **Antifoaming Agents- Olive Oil, Sunflower Oil, Silicones, Fatty acids, etc.

5. **Sterilization Procedures:**

   a) At laboratory scale- Autoclave at 121.6 ^{0}C temperature & at 15 psi pressure for 20-25 minutes.

   b) At large scale- Steam, directly, is used for sterilization at 15 psi pressure itself.

   ** The air used in the fermentation process is usually sterilized by filter sterilization.

6. **Aeration and Mixing:**

a) At lab scale- Aeration and Mixing- by using "Shaker".

(Aeration and mixing can be further augmented by use of baffle flask in place of normal Erlenmeyer flask.)

b) At industrial scale- Aeration- by "Sparger"

Agitation/Mixing- by "Stirrer"

7. **Microbial culture at Industrial Scale: -**

Fermenter (Bioreactor)

(i) These are vessels, which are used for large-scale growth of microorganisms under a controlled environment.

These are usually closed vessels with adequate arrangement for aeration, agitation, temperature and pH control, and drain or overflow vent to remove the cultured microbes along with their products.

The vessel is capable of being operated aseptically for a number of days. These are also equipped with sampling ports, which allow withdrawal of the fermentation broth at regular intervals while the fermentation is in progress.

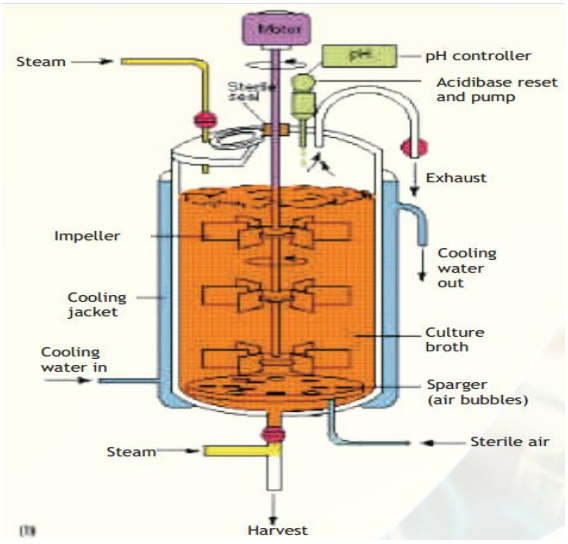


Fig- STB

(ii) Two types of bioreactors are:-

a) Stirred Tank Bioreactor

b) Bubble Column Reactor

(iii) **Important Parts of an STB:-**

pH controller,

Stirrer, Cooling Jacket,

Samplig Port,

Sparger

8. Types of Microbial Culture

Batch Culture	**Fed Batch Culture**	**Continuous Culture**
The nutrient medium is provided all at once at the start of the fermentation process.	Continuously or sequentially fed with fresh medium without removing the growing culture.	Fresh Culture medium is added accompanied with the removal of an equivalent amount of spent culture medium with cells.

9. Measurement and Kinetics of Microbial Growth

(i) Measurement of microbial growth

-Determination of dry weight of cells

-Turbidimetric Measurement

-Determination of Wet Weight of cells

-Use of Coulter Counter

-Viable Plate Count

(ii) Kinetics of microbial growth

Kinetics of microbial growth can be studied in relation to both ***cell number (N)*** and ***biomass concentration (X).***

Some important formulae regarding microbial growth kinetics are as follows: -

1. $\mu = 2.303 (\text{Log } X_t - \text{Log} X_0) / t$

2. $t_d = 0.693 / \mu$

3. $n = 3.3 (\text{Log } N_t - \text{Log } N_0)$

Here, **μ = specific growth rate,** **X_t = initial biomass**

X_t = biomass after time t, **t_d = doubling time**

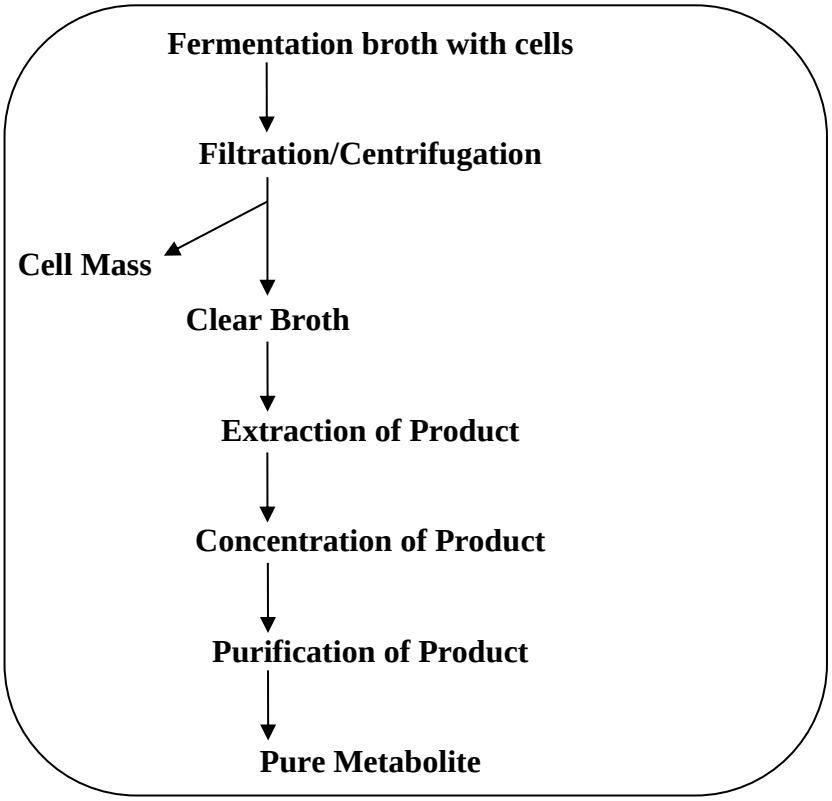
t = time taken, **n = no. of generations**

N_0 = initial cell number, **N_t = final cell number**

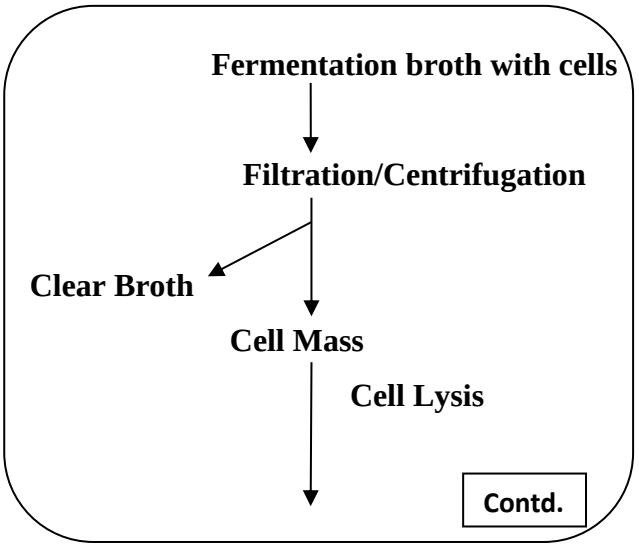
10. Isolation of Microbial Products

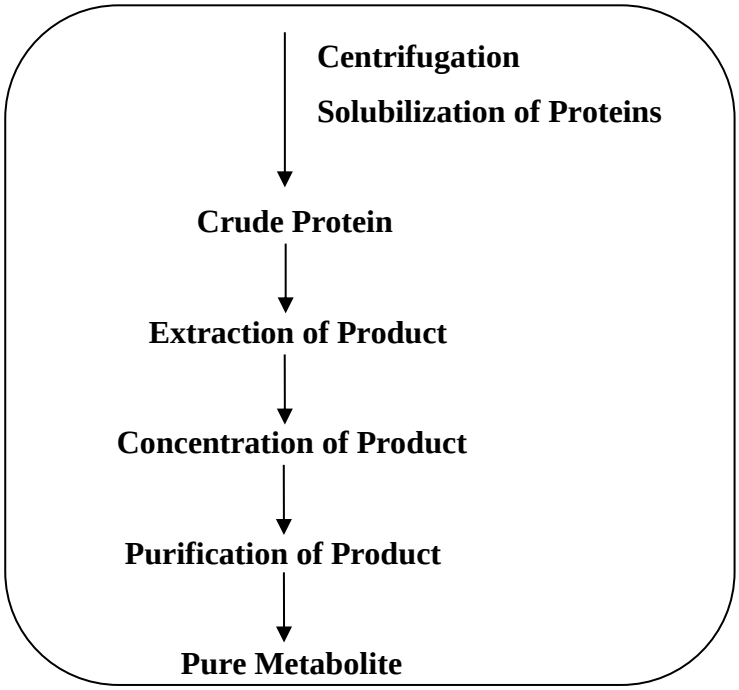
Once the fermentation is complete, it is necessary to recover the desired metabolite. Minimally, this will involve separation of the cells from the fermentation broth. But it may also include, purification of the metabolite with or without cell disruption; cell disruption will be necessary if the metabolite is intracellular. Such operations are referred to as downstream processing (DSP).

(a) Steps of DSP when the product is extracellular: -



(b) Steps of DSP when the product is intracellular:-





11. **Strain Isolation:**

To isolate the desired microbial strain from environmental sample (air/water/soil).

****Enrichment Technique:** The environmental sample containing the microbes (e.g. soil) is put in a nutritive medium and allowed to grow in shake cultures. The growth conditions (e.g. temperature) or nutrients in the medium are provided such that these favor the growth of microbes of our interest. This is called enrichment technique. The enriched culture can further be sub-cultured by taking a small inoculum and putting it into fresh medium. In this way, the growth of the desired organisms improves successively.

12. Strain Improvement:

Stain improvement involves making necessary changes in the characteristics of the isolated desired strain so that it can produce enhanced quantities of the metabolite of interest at commercial level.

Techniques used for strain improvement: -

(a) Classical Genetics- Mutant Selection Technique

(b) Genetic Engineering Technique

13. **Strain Preservation:**

Once a strain producing a novel or desired product has been obtained, it must be appropriately preserved for future use. 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.

Methods of Strain Preservation:

(i) Storage on Agar

(ii) Storage in Liquid Nitrogen

(iii) Freeze Drying/Lyophilization

14. **Culture Collection Centers: -**

Cultures may be deposited to culture collection centers.

These centers safely maintain cultures for years. The cultures are also made available to prospective investigators.

Examples:

ATCC- American Type Culture Collection, USA

NCIB- National Collection of Industrial Bacteria, UK

MTCC- Microbial Type Culture Collection & Gene Bank, Chandigarh, India

NBAIM- National Bureau of Agriculturally Important Microorganisms, UP, India

15. Applications of Microbial Culture Technology: -

1	Production of Whole Microbial cells (for food, vaccines)
2	Production of Primary Metabolite (acids, alcohol)
3	Production of Secondary Metabolite (antibiotics)
4	Biotransformation Reactions (enzymatic, steroid)
5	Utilization of Microbial Metabolism (bioleaching, biodegradable waste treatment)
6	Production of Recombinant Protein (therapeutic proteins)

16. Microbial species used for producing commercial products: -

Micro-organism	**Product**
Saccharomyces cerevisiae	Ethanol
Aspergillus niger	Citric Acid
Penicillium chrysogenum	Penicillin
Streptomyces griseus	Streptomycin
Corynebacterium glutamicum	L-Lysine
Propionibacterium shermanii	Vitamin B12
Aspergillus oryzae	Amylases
Leuconostoc mesenteroides	Dextran
Escherichia coli	Insulin, growth hormones and Interferon
Saccharomyces cerevisiae	Hepatitis B surface antigen
Alcaligenes eutrophus	Poly-3-hydroxybutyrate (PHB)

MIND MAP

MULTIPLE CHOICE QUESTIONS

1. The growth medium, which is prepared using pure chemicals such that full chemical composition of the medium is known, is called as: -
   A. Synthetic Medium
   B. Semi Synthetic Medium
   C. Commercial Medium
   D. Semi Soild Medium

2. Which of the following considerations is ***undesirable*** with regard to the selection of raw material(s) for the growth medium to be used at commercial stage microbial production process?
   A. Should yield maximum product or biomass per gram of the substrate used
   B. Should be of superior quality irrespective of the cost
   C. Should cause minimum problems during preparation and sterilization
   D. Give minimum problems during production process particularly aeration, agitation, extraction and purification of the product.
3. Which one of the following is ***not*** an antifoaming agent used in microbial culture media?
   A. Fatty Acids
   B. Olive Oil
   C. Silicone
   D. Iron
4. Which of the following parts of the stirred tank bioreactor is primarily involved in aeration?
   A. Cooling Jacket
   B. Sampling Port
   C. Sparger
   D. Stirrer
5. The apparatus named as incubator shaker-
   A. provides constant temperature and facilitates mixing
   B. provides constant temperature and facilitates aeration
   C. facilitates both mixing and aeration
   D. provides constant temperature, facilitates both mixing & aeration
6. Which of the following type of culture system would be most preferred for the commercial production of a recombinant protein?
   A. continuous culture system
   B. batch culture system
   C. fed batch culture system
   D. laboratory scale culture system
7. Which of the following methods of microbial growth measurement would be most preferable for measuring microbial growth?
   A. electronic particle count
   B. viable plate count

C. measurement of dry weight

D. turbidity measurement

8. If one cell of *Escherichia coli*, put into a nutritive medium, divides every 20 minutes, after 5 hours of such growth, the number of *E. coli* cells in the culture vessel would be-

A. 2^{12} B. 2^{5} C. 2^{10} D. 2^{15}

9. What would be the doubling time (t_d) of a bacterial population in which the number of bacteria increases from 10^4 cells/ml to 10^7 cells/ml during four hours of exponential growth?

A. 20 minutes B. 24 minutes

C. 22 minutes D. 30 minutes

10. Which of the following methods can **NOT** be used for separation of biomass from fermentation broth during downstream processing?

A. Flocculation B. Centrifugation

C. Membrane Filtration D. Ultrasonication

11. Which of the following culture collection centers is located in ***India***?

A. ATCC B. MTCC

C. DSM D. NCIB

12. What is the meaning of the term "metagenome"?

A. genome isolated from cultured microbial cells of a single species

B. genome isolated from cultured microbial cells of diverse species together

C. genome isolated from cultured plant or animal cells

D. genome isolated directly from an environmental sample

13. Which of the following modules can be installed in rDNA so as to ensure that production of recombinant protein does not occur until required?

A. strong promoter

B. signal sequence

C. regulatory switch

D. marker gene

14. Which of the following is **NOT** a method of microbial strain preservation?

A. Storage on Agar B. Storage in liquid nitrogen

C. Enrichment technique D. Lyophilization

15. Identify the correct match from the following:

S. No.	Microorganism	S. No.	Product

1	*Saccharomyces cerevisiae*	a	Citric acid
2	*Leuconostoc mesenteroides*	b	ethanol
3	*Alcaligenes eutrophus*	c	dextran
4	*Aspergillus niger*	d	Poly-3-hydroxybutyrate (PHB)

A. 1-b, 2-c, 3-d, 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**	B
2	B	**10**	D
3	D	**11**	B
4	C	**12**	D
5	D	**13**	C
6	A	**14**	C
7	B	**15**	A
8	D		

PREPARED BY: MR ABHINAV GOYAL

PGT BIOTECHNOLOGY, K V KHANAPARA, GUWAHATI

CHAPTER 5: PLANT CELL AND TISSUE CULTURE

GIST OF THE CHAPTER

Totipotency: The ability of a plant cell to regenerate into whole plant.

- **All plant cells are totipotent in nature.**
- **Gottlieb Haberlandt, is regarded as 'Father of Plant Tissue Culture'**

Basic technique culture involves the following steps:

- 1. Selection of suitable **explants** (any healthy excised plant part used to initiate plant cell culture).
- 2. Surface sterilization of the explants by disinfectants (e.g. sodium hypochlorite).
- 3. Inoculation (transfer) of the explants onto the suitable nutrient medium
- 4. Growing the cultures in the growth chamber or plant tissue culture room having the appropriate physical conditions [artificial light (16 h photoperiod), temperature (~26 C) and relative humidity (50-60%)].
- 5. Regeneration of shoots from cultured plant tissues and their elongation (cytokinin).
- 6. Rooting of regenerated shoots on rooting medium (auxin).
- 7. Transfer of plants to the transgenic green-house.

Nutrient media

- **An optimum pH (usually 5.7) is also very important.**
- The most extensively used nutrient medium is **MS medium.**

Types of cultures

1. **Organ culture:**

- It deals with the culture of the isolated organs (like roots) under laboratory conditions *(in vitro), and different names are given depending upon the organ utilized for the* culture.

2. **Explant culture:**

- The culture of plant parts (explants) is known as explant culture.
- The explants can be any part of the plant like the piece of stem, leaf, cotyledon, hypocotyls, etc.
- The explant cultures are generally used to induce callus for plant regeneration.

3. Callus culture:

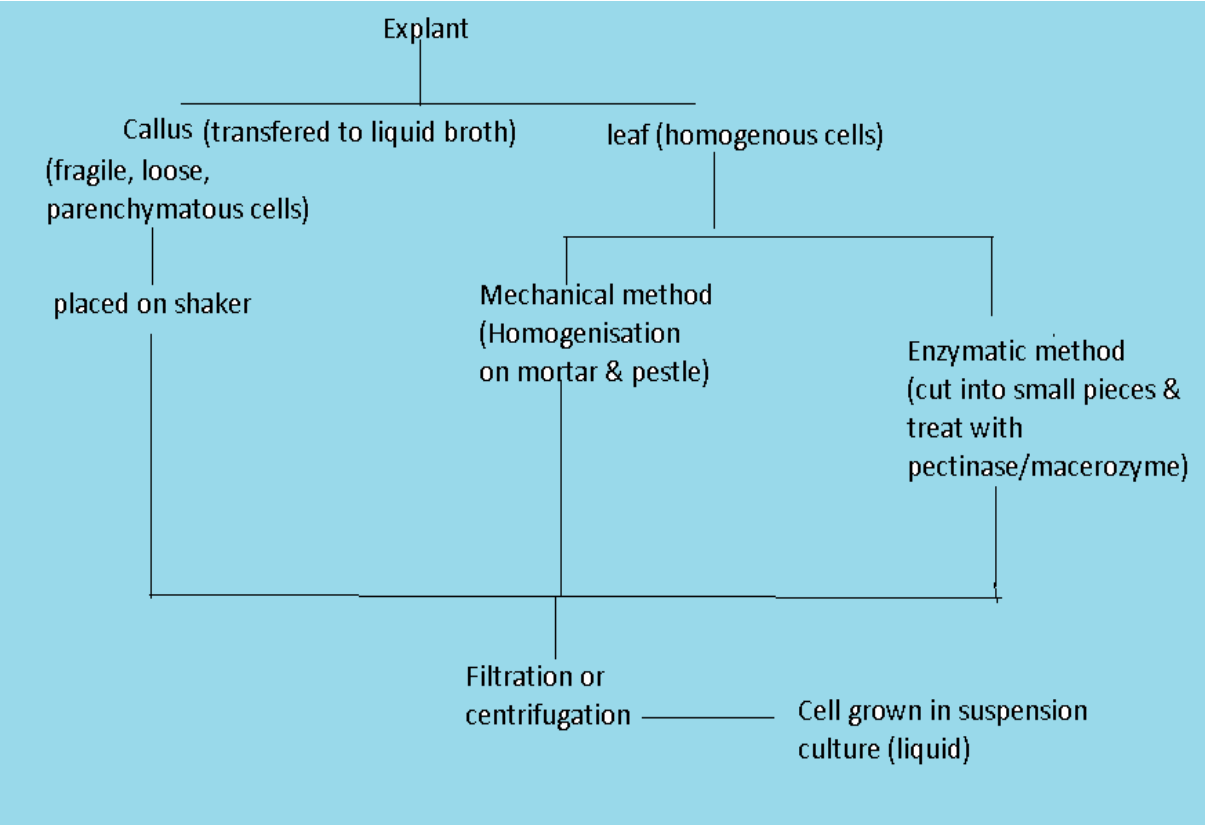
- **Callus refers to an unorganized mass of cells, which are generally** parenchymatous in nature.

Callus cultures are used for:

- plant regeneration (organogenesis & SE).
- preparation of the single cell suspensions and **protoplasts (cell without cell wall).**
- genetic transformation studies.

4. Cell suspension cultures:

- **Single cells can be isolated from either callus or any other part of the** plant (e.g. leaf) and cultured in liquid medium.
- 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.
- Once the cells have been isolated, they may be cultured by **batch cultures or continuous cultures.**



The cell suspension cultures can be used for:

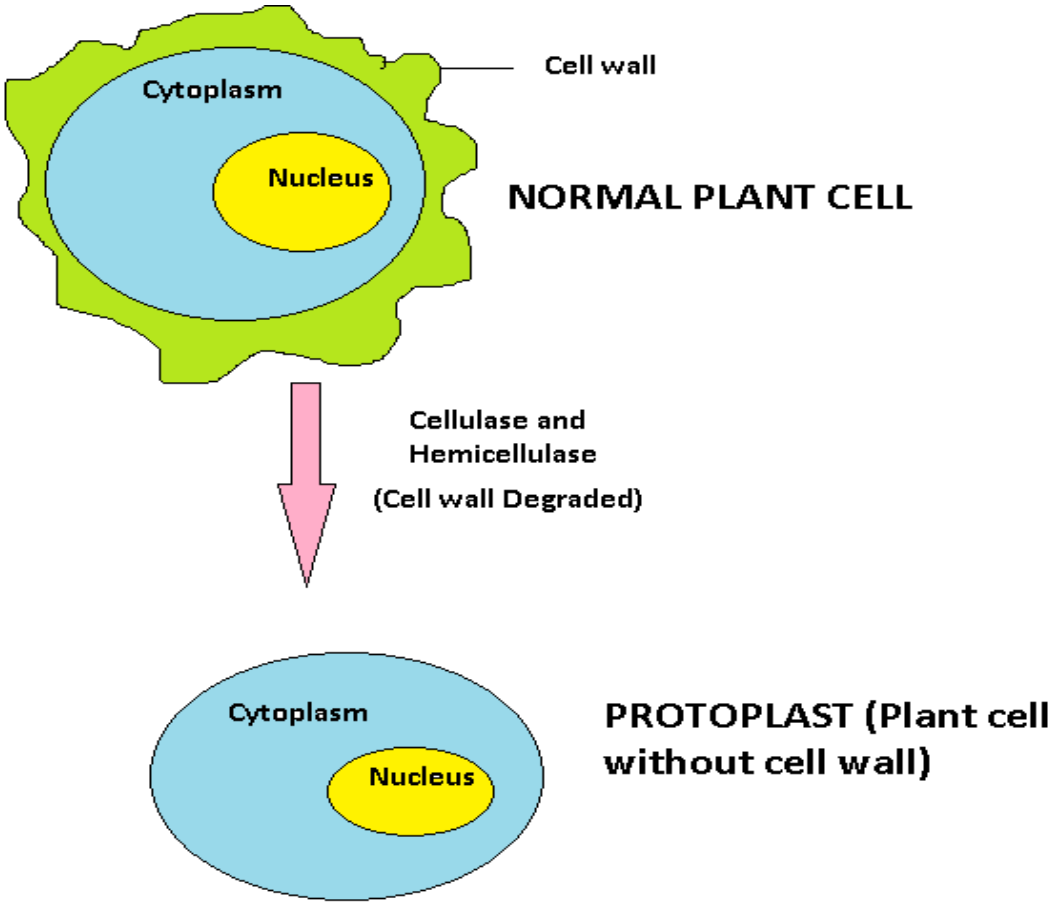
- induction of somatic embryos/shoots.
- *in vitro mutagenesis and mutant selection.*
- genetic transformation.
- production of secondary metabolites.

5. **Mass cell culture:**

- It involves the large-scale culture of cells in specially designed 'plant bioreactors', which essentially do not have a stirrer (as plant cells are shear sensitive).

6. Protoplast culture:

- **Protoplasts are plant cells without cell wall and can be isolated** from a variety of plant tissues by enzymatic method using cell wall digesting enzymes (cellulases, hemicellulases and pectinases).

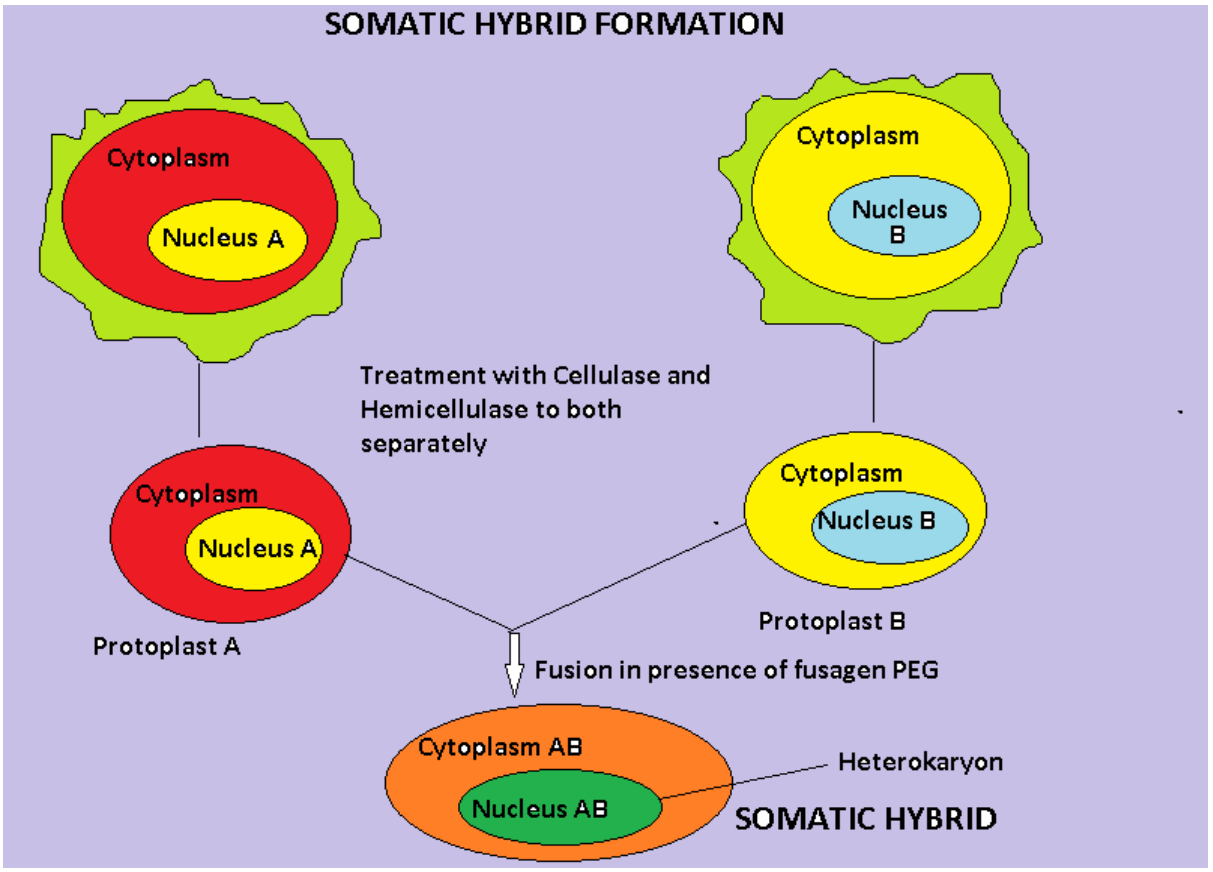


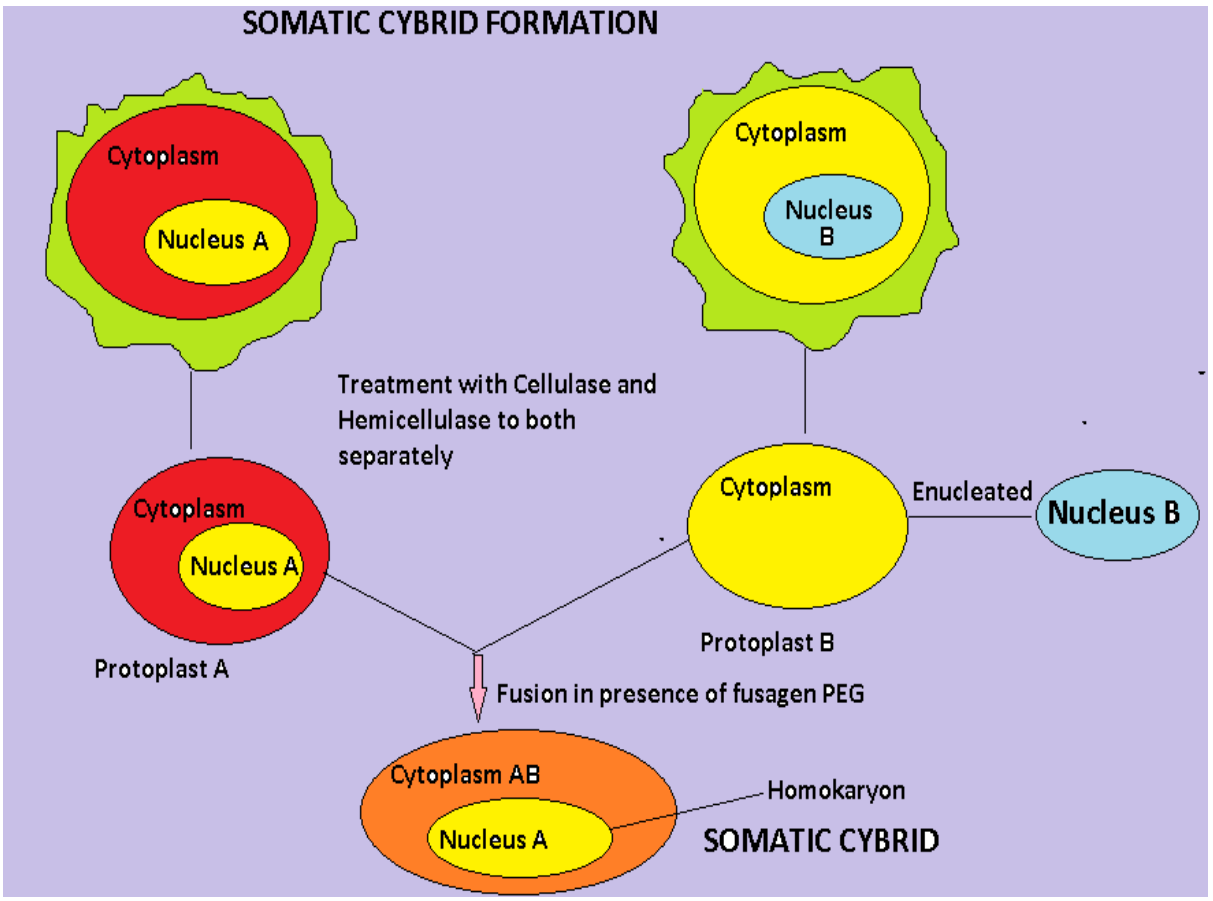
As the protoplasts lack cell wall, they can be utilized for many purposes such as:

- Various biochemical and metabolic studies.
- Fusion of two somatic cells to produce somatic hybrids.
- Fusion of enucleated and nucleated protoplasts to produce cytoplasmic hybrids (Cybrids).
- Genetic transformation.

Protoplast fusion:

- **The protoplasts from two different plant genotypes can be fused in the** presence of fusogenic agents like polyethylene glycol (PEG - most widely used and most successful method for protoplast fusion) or by electro-fusion.
- The hybrid cells **(heterokaryons)** can be selected by various methods such as the use of different antibiotic markers or fluorescent dyes for two different protoplasts.


SOMATIC HYBRID FORMATION
Cytoplasm
Nucleus A
Cytoplasm
Nucleus B
Treatment with Cellulase and Hemicellulase to both separately
Cytoplasm
Nucleus A
Protoplast A
Cytoplasm
Nucleus B
Protoplast B
Fusion in presence of fusagen PEG
Cytoplasm AB
Nucleus AB
Heterokaryon
SOMATIC HYBRID


SOMATIC CYBRID FORMATION
Cytoplasm
Nucleus A
Cytoplasm
Nucleus B
Treatment with Cellulase and Hemicellulase to both separately
Cytoplasm
Nucleus A
Protoplast A
Cytoplasm
Enucleated
Nucleus B
Protoplast B
Fusion in presence of fusagen PEG
Cytoplasm AB
Nucleus A
Homokaryon
SOMATIC CYBRID

Plant Regeneration Pathways:

A) **Organogenesis means formation of organs like shoots from the cultured explants**

Miller and Skoog experimentally proved that formation of shoot or root first on the cultured tissue depends on the relative concentration of auxin and cytokinin.

B) In **somatic embryogenesis, the totipotent cells may undergo embryogenic pathway to form** somatic embryos **which can be grown to regenerate into complete plants.**

Applications of Cell and Tissue Culture

1. **Micropropagation:**

- 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).**
- Thus, tissue culture method of plant propagation, known as **'micropropagation' can be** used to overcome many problems associated with vegetative propagation.

2. Virus Free Plants

- Most of the crops plants, particularly vegetatively propagated plants are systemically infected with viruses.

Apical meristems are devoid of viruses because:

- They are fast dividing and don't allow the viral propagation inside them.
- They are devoid of vascular bundles which spread infection in plants.

3. Artificial Seeds

- 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.
- Artificial seeds are produced either by **encapsulating** the somatic embryos in a protective coating, i.e., **calcium or sodium alginate** beads or by desiccating the somatic embryos with or without coating.

4. Embryo Rescue

- It is very difficult to produce hybrids in case of inter-specific and inter-generic crosses (crosses between distantly related plants) because of abnormal development of the endosperm, which causes premature death of the hybrid embryo and leads to sterile seeds **(embryo-endosperm incompatibility)**.
- The embryo from such sterile hybrid seeds can be excised at an appropriate time and cultured on a suitable nutrient medium to produce novel hybrids which is otherwise not possible. This is known as embryo rescue.

5. Haploid

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

- It enables raising plants expressing traits that are otherwise recessive.

- The genetically homozygous diploid plants, which serve as parents in cross breeding can also be produced by diploidization of haploid plants using **colchicine** chemical (arrest cell division at metaphase).
- Allows In-Vitro Mutagenesis.

6. Triploid Culture 3n

- **Endosperm Culture:** The endosperm is a triploid tissue and an excellent material to produce triploid plants by culturing endosperm tissue. Triploid plants usually show seed sterility or seed lessness, which is desirable in crops like citrus, apple and pear.

7. Somatic Hybrid & Cybrid Formation

- The **somatic cell hybridization (also known as parasexual hybridization)** offers an excellent alternative for obtaining distant hybrids with useful agronomic traits (known as **somatic hybrids** or **parasexual hybrids),** which would never be formed in nature through sexual fertilization.
- It is also possible to produce cytoplasmic hybrids **(cybrids)** through protoplast fusion in which the genomes of one of the partners is lost.
- Alternatively, the isolated and purified organelles - chloroplasts or mitochondria from one species can be fused with the recipient protoplasts from a different plant species (known as **organelle transfer or organelle uptake)** to transfer useful cytoplasmic traits **like herbicide tolerance and cytoplasmic male sterility.**

8. Production of secondary metabolites

- **Primary metabolites:** Metabolites which are required for further growth and development of cells.
- **Secondary métabolites:** Metabolites which are not required for further growth and development of cells but be secreted by cells for defense against pests and pathogens as well as feeding by animals. Ex: alkaloids, resins, tannins, latex, are of immense use in medicine.

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

9. **Somaclonal variation**

- It has been observed that the long-term callus and cell suspension culture and plants regenerated from such cultures are often associated with chromosomal variations **(somaclonal variation).**
- It is this property of cultured cells that finds potential application in the crop improvement and in the production of mutants (e.g., disease resistance in potato).

In vitro plant germplasm conservation

- **Germplasm** refers to the sum total of all the genes present in a crop and its related species.
- Conventional methods (e.g. seeds, vegetative propagules, etc. and it is known as ***in vivo gene banks)*** as well as non-conventional methods, i.e**.** cell and tissue culture methods (known as ***in vitro gene banks)*** are used.
- **Freezing storage or Cryopreservation** - this utilizes the long-term preservation of cells and tissues (e.g. shoot tips, axillary buds, meristems, somatic embryos, etc.) at ultra-low temperature (-196 ^{0}C, i.e. in liquid nitrogen) for definite time by using cryoprotectants (e.g. dimethylsulfoxide, glycerol, proline and mannitol). The cells and tissues can be recovered after thawing, and can be used for regeneration of plants.

Transgenic plants with beneficial traits

Stress tolerance

- **Biotic stresses**: (viral, bacterial, fungal pathogens, nematodes, insect / pests and weeds)
- **Abiotic stresses**: (salinity, drought, extreme temperatures, nutrient deficiency,)

Biotic stress tolerance

A) Insect / Pest Resistance

B) Microbial

- Fungal
- Bacterial
- Viral

1. **Pest / Insect Resistance:**

- The transgenic technology provides an alternative and innovative method to improve pest control management which are eco-friendly, effective, sustainable and beneficial in terms of yield.
- The first genes available for genetic engineering of crop plants for pest resistance were *Cry genes (*popularly known as *Bt* genes*)* from bacterium *Bacillus thuringiensis.*
- Cotton: cotton bollworms
- *Bacillus thuringiensis*: *cry genes (*multigene family): toxic to different insects.
- Cry 1Ac: delta endotoxin: create pores in the gut of insect in an alkaline-> insect gets killed.
- Cotton plant→ *cry1Ac* →Bt Cotton→ Cry 1Ac toxin (inactive)→Insect Resistant.

2. **Virus resistance:**

- There are several strategies for engineering plants for viral resistance, and these utilizes the genes from virus itself (e.g. the viral coat protein gene).
- The virus-derived resistance has given promising results in a number of crop plants such as tobacco, tomato, potato, alfalfa and papaya.

Fungi and bacteria:

- Plants respond to pathogens by inducing a variety of defense responses like pathogenesis-related proteins (PR proteins), enzymes that degrade/destroy fungal cell wall, antifungal proteins and compounds, phytoalexins, etc.
- Several transgenic crop plants showing increased resistance to fungal pathogens are being raised with genes coding for the different compounds mentioned above.

Abiotic stress tolerance

1. **Herbicide tolerance:**

- Farmers apply herbicides/weedicides (e.g. glyphosate) for the eradication of weeds in the fields, but the main problem with this is the development of herbicide tolerance by weeds.
- There are several biotechnological strategies for weed control, but the most commonly employed approach is the over-production of herbicide target enzyme (usually in the chloroplast) in the plant, so that it becomes insensitive to the herbicide.

Herbicides can act an inhibitor by binding to a target enzyme and blocking its catalytic activity thus killing the plant cell. This can also lead to reduction in yield of the crop plants.

- Strategies for herbicide resistance:

a) Over production of the metabolite.

b) Slight structural alteration of the target enzyme.

c) Degradation of the herbicide in the transgenic plant.

d) The popular example for such an approach is the introduction of a modified gene from an *Agrobacterium* species that encodes for a resistant form of the herbicide target enzyme into crop plants for tolerance against the most extensively used herbicide **glyphosate (sold as Roundup)** and is effective against many weeds.

e) **Roundup Ready GM** crop plants such as canola, soybean, corn and cotton tolerant to glyphosate has already been commercialized.

Other Abiotic Stress Factors

- Plants have evolved many types of adaptations to cope with abiotic stress conditions like the production of the stress-related osmolytes like sugars (e.g. trihalose and fructans), sugar alcohols (e.g. mannitol) and amino acids (e.g. proline), glycine betaine, and certain proteins (e.g. antifreeze proteins).
- Transgenic plants have been developed which over-express the genes for one or more of the above-mentioned compounds. Such plants have shown increased tolerance to environmental stresses.

Delayed fruit ripening:

- The gas hormone, ethylene is involved in the regulation of fruit ripening. Therefore, ripening can be slowed down by blocking or reducing ethylene production.
- This can be achieved by introducing ethylene forming gene(s) in a way that will suppress its own expression in the crop plant. (Antisense RNA technology).
- Fruits from such plants ripen very slowly (however, they can ripen by ethylene application) and are very important for export to longer distances without spoilage as they show longer-shelf life due to slow ripening.
- The notable example of this kind is the ***'Flavr Savr' transgenic tomatoes which*** were commercialized in U.S.

Male sterility and fertility restoration:

- 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 bacterial gene from *Bacillus amyloliquefaciens that encode an enzyme **barnase***, which is an RNA hydrolyzing enzyme that inhibits pollen formation if, the expression of this gene specifically in the tapetal cells of anther using **tapetal-specific promoter (e.g. TA29) to restrict its activity** only to the cells involved in pollen production.
- Male fertility can be restored by introducing another gene from the same bacterium under the control of TA29, whose product **barstar** (protein) tightly bind with RNase, so that the normal pollen are formed.

- This **barnase/barstar** system was successfully utilized in producing male sterile/restorer lines in number of crops, particularly mustard for hybrid production.

Nutrient quality

Vitamin A:

- In a remarkable example of genetic engineering, Prof. Ingo Potrykus and Dr. Peter Beyer developed genetically engineered rice (popularly known as **'Golden Rice'),** which is enriched in pro-vitamin A (beta-carotenoids) by introducing three genes involved in the biosynthetic pathway for carotenoid under the control of endosperm-specific promoter, so that gene products (enzymes) are synthesized in the rice endosperm.
- The seeds of Golden Rice are yellow in color because of pro-vitamin A is produced in the entire grain.

Seed protein quality:

- The nutritional quality of cereals and legumes are limited because of deficiency of the essential amino acids, i.e. lysine in cereals, and methionine and tryptophan in pulses.
- Two genetic engineering approaches have been used to improve the seed protein quality.
- In the first case, a transgene (e.g. gene for protein containing Sulphur rich amino acids was introduced into pea plant (which is deficient in methionine and cysteine, but rich in lysine) under the control of seed-specific promoter.
- In the second approach, the endogenous genes are modified, so as to increase the essential amino acids like lysine in the seed proteins of cereals.

Edible vaccines:

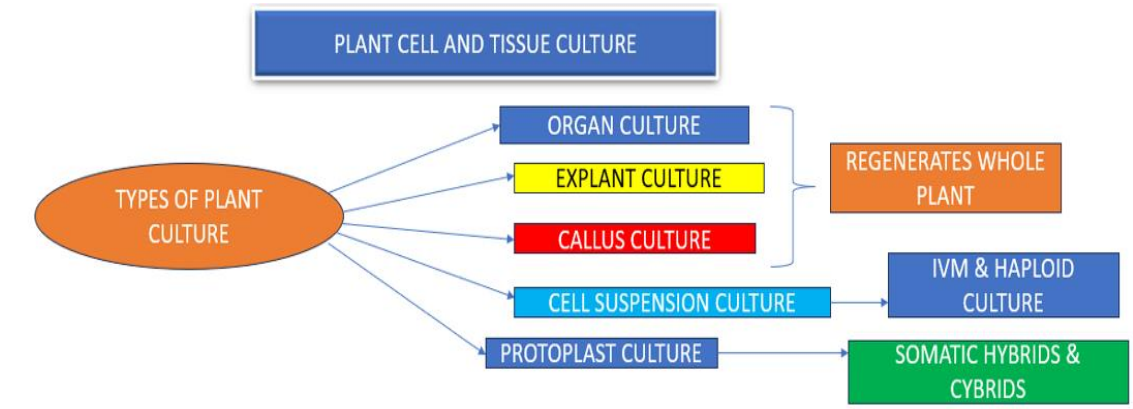
- The genes encoding antigenic proteins can be isolated from the pathogens and expressed in plants, and such transgenic plants or their tissues producing antigens can be eaten for vaccination/immunization **(edible vaccines).**
- **The expression of such antigenic** proteins in crops like banana and tomato are useful for immunization of humans since banana and tomato fruits can be eaten raw. In case of animals, such genes can be expressed in crops like alfalfa and other forage/fodder crops, which are suitable for vaccination.

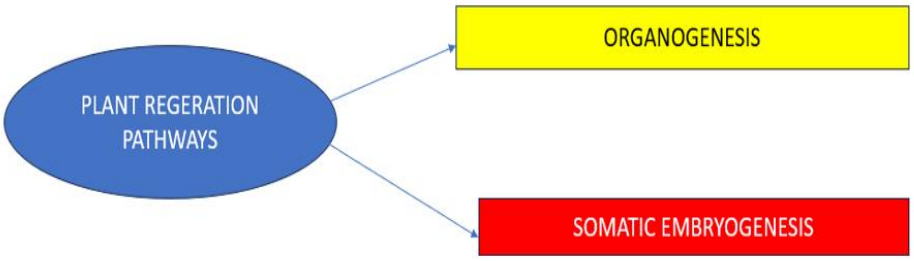
Biodegradable plastics

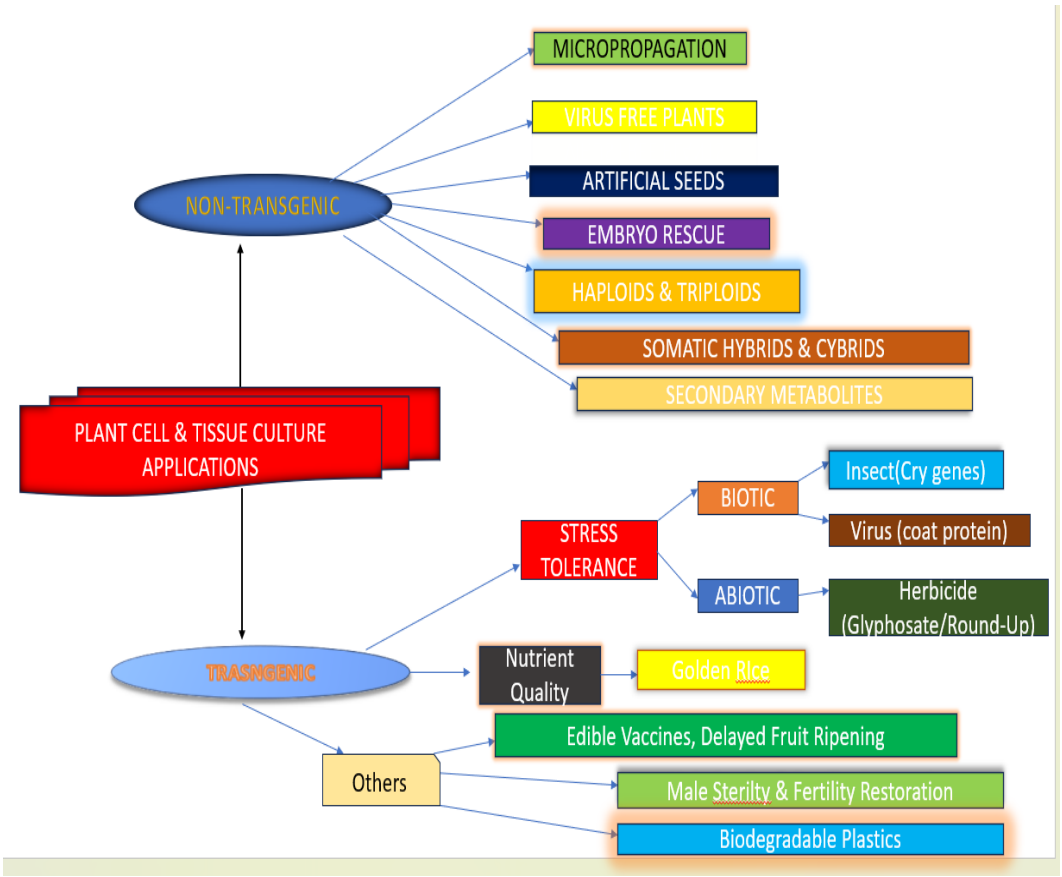
- The biodegradable plastic, e.g. polyhydroxybutyrate (PHB) is being produced commercially by fermentation with the bacterium *Alcaligenes eutrophus.*
- Transgenic plants can be used as factories to produce PHB. The genetically engineered *Arabidopisis thaliana* plants with the three genes involved in PHB synthesis from *A. eutrophus* produced PHB globules exclusively in their chloroplasts without effecting plant growth and development**.**

The large-scale production of PHB may be easily achieved in tree plants like populus, where PHB can be extracted from leaves. Industry has already begun to explore the production of biodegradable plastics from transgenic plants.

MIND MAP


PLANT CELL AND TISSUE CULTURE
TYPES OF PLANT CULTURE
ORGAN CULTURE
EXPLANT CULTURE
CALLUS CULTURE
REGENERATES WHOLE PLANT
CELL SUSPENSION CULTURE
IVM & HAPLOID CULTURE
PROTOPLAST CULTURE
SOMATIC HYBRIDS & CYBRIDS


PLANT REGERATION PATHWAYS
ORGANOGENESIS
SOMATIC EMBRYOGENESIS


MICROPROPAGATION
VIRUS FREE PLANTS
ARTIFICIAL SEEDS
NON-TRANSGENIC
EMBRYO RESCUE
HAPLOIDS & TRIPLOIDS
SOMATIC HYBRIDS & CYBRIDS
SECONDARY METABOLITES
PLANT CELL & TISSUE CULTURE APPLICATIONS
Insect(Cry genes)
BIOTIC
STRESS TOLERANCE
Virus (coat protein)
ABIOTIC
Herbicide (Glyphosate/Round-Up)
TRASNGENIC
Nutrient Quality
Golden Rice
Edible Vaccines, Delayed Fruit Ripening
Others
Male Sterilty & Fertility Restoration
Biodegradable Plastics

MULTIPLE CHOICE QUESTIONS

1. Which of these is considered to be the era of 'Second Green Revolution'?
   A. The first phase of tissue culture when attempts were made to culture isolated plant organs.
   B. The second phase when extensive studies were done to develop suitable nutrient media.
   C. Era of developing transgenic plants
   D. Both a and b.
2. Which of these chemicals will be used for surface sterilization of explants:
   A. Sodium Hypochlorite
   B. Polyethylene Glycol
   C. Calcium Alginate
   D. DMSO.
3. The gene which was used to produce insect-resistant cotton plant was taken from _____
   A. *Anabaena azollae*
   B. *Agrobacterium tumefaciens*
   C. *Bacillus thuringiensis*
   D. *Bacillus subtilis*
4. Tobacco plant was made resistant to TMV by:
   A. introducing T-DNA of Agrobacterium
   B. introducing a gene of virus coat protein
   C. Introducing meristematic activity in somatic cells.
   D. Applying new generation pesticides to the crops.
5. Which of these is not a plant tissue culture media:
   A. Tryptic Soya Broth
   B. Murashige and Skoog
   C. LS
   D. B5
6. What are the strategies of creating a herbicide tolerant plant:
   A. Over Expression of the target enzyme
   B. Alteration of the target enzyme
   C. Destruction of the herbicide inside cells of crop plants.
   D. All of these.
7. Which type of culture will be done for in-vitro mutagenesis in plants:

A. Callus Culture

B. Organ Culture

C. Explant Culture

D. Cell suspension culture.

8. Which of these is not a function of auxins in plant tissue culture:

A. Root initiation

B. Shoot Initiation

C. Callus Induction

D. Somatic Embryogenesis induction

9. Which stage of somatic embryo is used for creating artificial seeds:

A. Torpedo

B. Heart

C. Globular

D. All of these

10. Which of the following is the correct expansion of RAPD?

A. Rapid Amplified Polymorphic DNA.

B. Randomly Amplified Polymorphic DNA

C. Restriction Fragment Length polymorphism

D. Amplified Fragment Length Polymorphism

11. A researcher wants to create a virus resistant plant, which of these strategies he may adopt:

A. Apical Meristem Culture

B. Axial Meristem Culture

C. Coat Protein Mediated Resistance

D. Somaclonal Variation

12. Which of the following is a herbicide tolerant plant:

A. Bt-Cotton

B. Round-Up Ready Canola

C. Flavr-Savr Tomato

D. Coat Protein enabled transgenic plants.

13. Organellar transfer into recipient protoplasts can done by somatic Cybridization for producing

A. herbicide tolerant plants

B. Cytoplasmic male sterile plants

C. Both a and B

D. Secondary metabolites

14. Which of these is produced by culturing *Lithospermum erythrorhizon* plant cell culture which can be used as red pigment in lipsticks.
    A. Taxol
    B. Shikonin
    C. Vincristine
    D. Quinine
15. Which of these is not used for cryopreservation of plant cells or tissues:
    A. Proline
    B. DMSO
    C. Serum
    D. Mannitol
16. A farmer wants to grow sugarbeet plants with improved oil production, with which of the genes the plant has been transgenic and who is the developer?
    A. EPSP Synthase by Monsanto
    B. Bt CryIII by Calgene
    C. CryIAc by Cornell University
    D. Gmfad2-1 by Dupont
17. Choose the correct pair of gene from its source organism for creating male sterile plants:
    A. *Barnase-Bacillus thuringiensis*
    B. *Barstar-Bacillus amyloliquefaciens*
    C. *PHB-Bacillus thuringiensis*
    D. *Barnase- Bacillus amyloliquefaciens*
18. Polyhydroxybutyrate globules produced in chloroplasts of transgenic *Arabidopsis* plants are used for making
    A. Vaccines
    B. Biodegradable Plastics
    C. Weed tolerant plants
    D. Insect resistant plants.
19. A researcher is trying to produce excess secondary metabolites produced in a plant. What strategy he can apply?
    A. He can infect the plant with *Agrobacterium tumefaciens*
    B. He can infect the plant with *Agrobacterium rhizogenes*
    C. He can infect the plant with *Bacillus amyloliquefaciens*

D. He can infect the plant with *Bacillus amyloliquefaciens*

20. Which of these is **NOT** a concern with transgenic plants:

A. High Yield

B. Safety of GM food by human and animal

C. Effect of GM crops on non-target insects

D. Effect on biodiversity

ANSWER KEY OF MCQ FROM PLANT CELL AND TISSUE CULTURE

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
C	A	B	B	A	D	D	B	A	B	C	B	C	B	C	D	D	B	B	A

CHAPTER 6 : ANIMAL CELL CULTURE

GIST OF THE CHAPTER

- The growth rate of animal cells is relatively slow and usually require 48 to 72 hours to divide.
- The animal cell culture became a routine laboratory technique in 1950s after **George Gay established the first human cell line (HeLa) from cervix cancer** that led to several important discoveries in medical sciences.

Features of animal cell growth in culture:

1) **Mortality**: A normal animal cell can survive for 2-3 generations under culture conditions.
2) **Contact Inhibition**: When the animal cells come in contact with each other or with the surface of the container they stop growth (Monolayer with very low density).
3) **Anchorage Dependence/ Adherence**: Animal cells need a matrix on which they can grow under culture condition.

Types of Animal Cell Cultures:

1) **Primary Cell Cultures:**

- Cells are dissociated from the parental tissue by mechanical (homogenization) or enzymatic methods.
- 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.
- The maintenance of growth of such cells under laboratory conditions is known as primary cell culture.
- *The drawbacks of primary culture* are that they are time consuming and require the use of live animals or fresh tissue.

Secondary Cell Cultures and Cell Lines

- Once the primary culture is subcultured, it is known as secondary culture or cell line.
- Subculturing or "splitting cells," is required to periodically provide fresh nutrients and growing space for continuously growing cell lines.
- **Rous and Jones were first to introduce proteolytic enzyme trypsin for the** subculture of adherent cells.

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)

Animal Cell Culture Requirements

Temperature:

- The mammalian cells are grown in incubators maintained at 37°C.

pH

- The regulation of extra-cellular and intra-cellular pH is essential for the survival of mammalian cells.
- Most media strive to achieve and maintain the pH between 7 and 7.4.
- Most media use the **Sodium bicarbonate (NaHCO3– CO2)** buffering system.
- The bicarbonate content of the medium neutralizes the effect of increased CO2.
- The increased $HCO3^-$ ion drives the equation above to the left until the equilibrium is reached at pH 7.4.

Osmolality:

- It preserves the membrane integrity of cells.

- 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 a final osmolality of around 300 mOsm.

Medium:

- Many of the media contain **phenol red as a pH indicator.**
- Highly acidic conditions turn the **phenol red into yellow while highly alkaline conditions turns the phenol red into pink color.**

Serum & Antibiotics

- Serum is one of the most important components of animal cell culture, as
- The peptide hormones or hormone-like growth factors that promote healthy growth are often derived from animal blood, such as **fetal bovine serum (FBS).**
- Serum is a source of various amino acids, hormones, lipids, vitamins, polyamines and salts containing ions such as calcium, chloride, ferrous, ferric, potassium etc. and it also supports cell proliferation and their attachment to culture vessels.

Vessels and Equipment required for Animal Cell Culture

- Cultures should be examined daily for their morphology, color of the medium and density of cells.
- The animal cells are usually grown and maintained in Petri dishes, Culture flasks or Multiwell plates of various shapes and sizes **at an appropriate temperature and gas mixture** (typically, 37°C, 5% CO2 for mammalian cells) in an incubator.
- Culture conditions vary widely for each cell type, and variation of conditions for a particular cell type can result in different phenotypes being expressed.

a) 5 % to 10 % CO2 Incubator:

- The CO2 incubator is designed to reproduce as closely as possible the environmental conditions of the living cells.
- The essential functions of the incubator are to maintain, the sterility of the chamber, a constant temperature, an atmosphere with a fixed level of CO2, high relative humidity (80%), pH maintenance.

b) Centrifuge:

- **A gentle braking action** helps prevent disruption of the separated bands of cells.

c) Inverted Microscope:

- In tissue culture vessels, for example a petri dish, the cells are present at the bottom with the culture medium above.
- The inverted microscope allows the cells at the bottom to be visualized because the optical system is at the bottom with the light source on top.

Methods of Gene Delivery into Cells

a) Calcium phosphate:

- Here HEPES-buffered saline solution is mixed with a calcium chloride solution containing DNA for transfection to form a fine precipitate of calcium phosphate with DNA.
- The suspension of the precipitate is then added to the monolayer of cells.
- The cells take up the calcium-phosphate- DNA complexes by endocytosis and express genes.

b) Lipofection:

- In this case, gene is transferred with the help of tiny vesicles of bipolar phospholipids that fuse with the cell membrane, releasing the DNA into the cytoplasm.

c) **Microinjection**

d) Electroporation

Applications of Animal Cell culture

a) Erythropoietin

- Erythropoietin (EPO) is a glycoprotein hormone that is involved in RBC production (erythropoiesis) and wound healing.
- EPO stimulates the bone marrow to produce more red cells and thereby increase the oxygen-carrying capacity of the blood.
- It is produced in response to hypoxia (shortage of oxygen) or anoxia (lack of oxygen) caused by anemia.
- EPO is useful in the treatment of certain types of anemia such as anemia due to cancer, chronic renal failure and treatment of AIDS.

b) Factor VIII: Hemophilia A is a common heritable genetic disorder where the body lacks the ability to produce Factor VIII required for blood clotting. Like EPO, factor VIII is also a glycoprotein and has been produced in CHO cells due to its large structure.

c) Factor IX: Hemophilia B or Christmas disease is the second most common type of bleeding disorder due to deficiency of factor IX. Recombinant Factor IX produced in CHO cells is used to treat hemophilia B.

d) tPA: Converts plasminogen into plasmin which dissolves blood clots. It is administered in case of ischemic stroke.

HYBRIDOMA TECHNOLOGY

- Invented by Kohler & Milstein.
- Produced by fusing B cells producing specific monoclonal antibodies with myeloma cells.
- Myeloma Cells provide immortality to mAb producing B cells.
- Selective HAT media is used.
- Monoclonal Ab are specific towards a specific epitope of an antigen.

APPLICATIONS OF HYBRIDOMA TECHNOLOGY

It produces mAb that can be used for diagnosing and treatment of various diseases.

OKT-3: A monoclonal Ab that is used as an immunosuppressive drug and is administered for reversal of acute graft rejection. It binds to CD3 antigen of T cells.

Therapeutic mAb - Herceptin

- Herceptin (trastuzumab) is a monoclonal antibody approved for therapy of early-stage breast cancer that is Human Epidermal growth factor Receptor 2-positive (HER2+).
- Herceptin works by attaching itself to HER2 receptors by blocking them from receiving growth signals. The result is impaired growth of breast cancer.

Stem Cell Technology

- **Self-Renewal:** Stem cells are characterized by their ability to renew themselves through mitotic cell division
- **Differentiation**: differentiate into a diverse range of specialized cell types.
- Stem cells are found in all multi cellular organisms.
- The field of stem cell research was established in 1960s by **Ernest McCulloch and James Till at the** University of Toronto.
- The two broad types of mammalian stem cells are: **embryonic stem (ES) cells that are isolated** from the inner cell mass of blastocysts, and **adult stem cells that are found in adult tissues.**
- The ES cells are **pluripotent** 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

ES Cell culture and its applications:

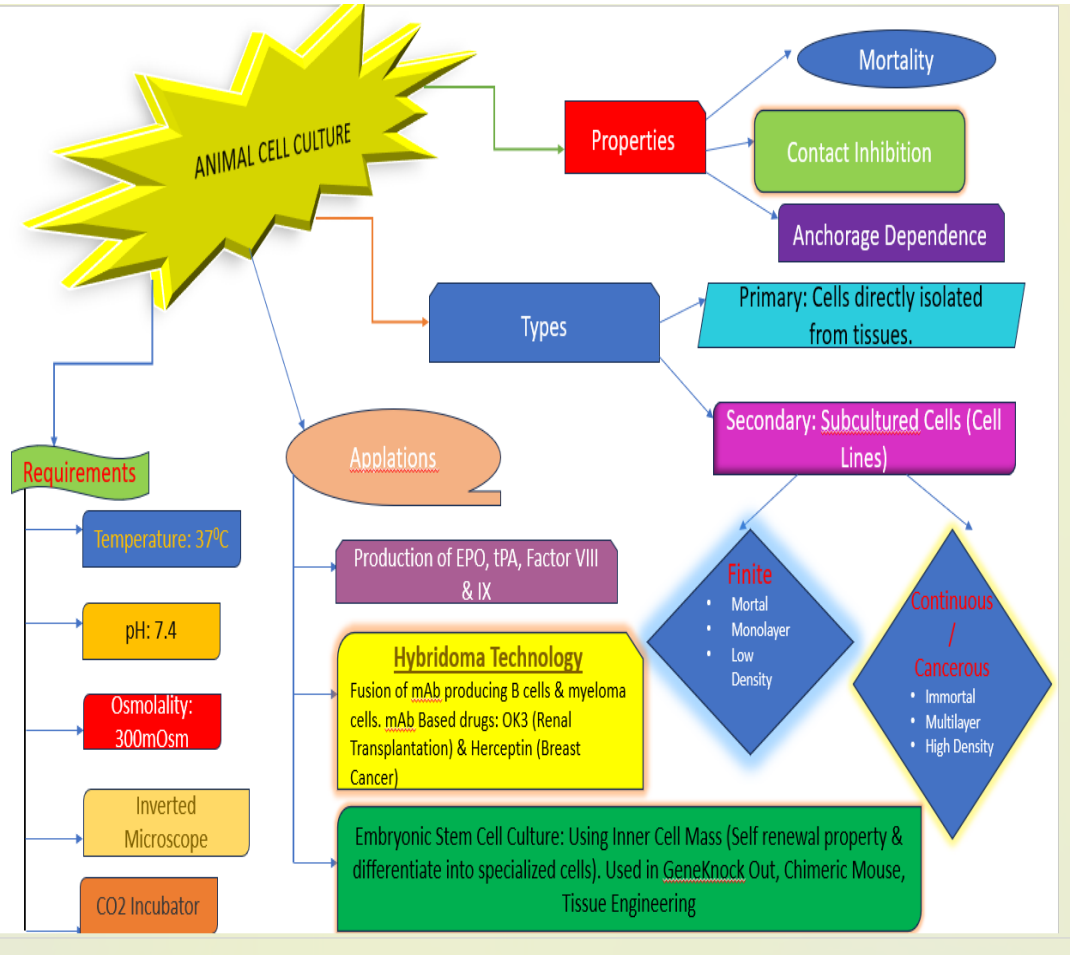
- The ES cells are cell lines derived directly from the inner cell mass of growing embryos without use of immortalizing or transforming agents.
- The **inner cell mass (lCM) of embryos** can be maintained in cell culture in the presence of irradiated fibroblast cells.

The stem cells:

- retain the characteristics of founder cells, even after prolonged culture and extensive manipulation.
- reintegrate fully into embryogenesis if transferred.
- 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.
- could maintain a stable euploid karyotype.
- could self-renew without differentiating in culture.

Gene Knockout: The process of deleting a gene from the genome of an organism to know its function. It can be achieved by homologous recombination.

MIND MAP



MULTIPLE CHOICE QUESTIONS

1. 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
2. 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

3. 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

4. Which of the following gene transfer methods in animal cells need HEPES buffered saline?

A. Lipofection

B. Microinjection

C. Electroporation

D. Calcium Phosphate mediated

5. 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

6. 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.

7. Almost all animal cell culture media are formulated to have a final osmolality of

A. 400 mOsm

B. 300 mOsm

C. 200 mOsm

D. 100 mOsm

8. Which amongst these was the first drug to be produced through mammalian cell culture?
   A. EPO
   B. tPA
   C. Factor VIII
   D. PDGF
9. Which of these human proteins is/are glycoprotein by nature and are produced in CHO cell lines:
   A. EPO
   B. Factor VIII
   C. tPA
   D. Both A and B
10. OKT-3 is a monoclonal body that is used for reversing acute graft rejection because:

A. It binds to CD-3 antigen of T cells thus blocking them.
B. It is produced by B cells
C. It is produced by hybridoma technology
D. It has therapeutic properties.

11. The first monoclonal antibody used for treatment of patients was:

A. Herceptin
B. OKT-3
C. Both A and B were used at the same time.
D. FSH

12. Which of the following pair of scientists were awarded with noble prize for inventing the Hybridoma technology:

A. Kohler and Milstein
B. Rous and Jones
C. McCulloh and Till
D. Smith and Nathans

13. 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.

14. 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

15. What is the principle behind Gene-Knock Out?

A. Homologous Recombination

B. Homozygous Relocation

C. Homologous Relocation

D. Homozygous Recombination

16. The first human cell line to be established from cervical cancer was:

A. CHO B. Cos-1 C. HeLa D. Both A and B

17. Which of the following properties is **NOT** a characteristic of finite cell lines:

A. High Density B. Contact Inhibition C. Adherence D. Mortality

18. Identify the correct order of subculturing 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.

19. Phenol Red would change to which colour if the media turns highly acidic?

A. Pink B. Yellow C. Orange D. No Change

20. Which of the following factors can be lethal to cells undergoing freezing during cryopreservation:

A. Formation of ice crystals

B. Alteration in electrolyte concentration

C. Dehydration or change in pH

D. All of these.

ANSWER KEY OF MCQ FROM ANIMAL CELL CULTURE

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
B	D	D	D	B	A	B	B	D	A	B	A	C	B	A	C	A	D	B	D

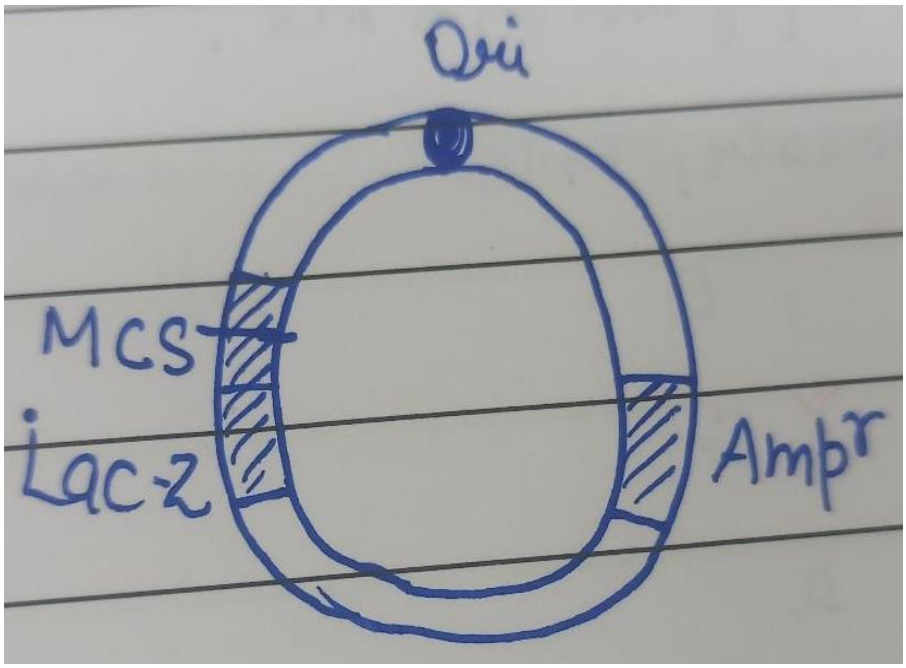
DEVELOPED BY: MRS ARPITA BASU

PGT BIOTECHNOLOGY, K.V NO.1 BHUBANESWAR (1ST SHIFT).

COMPETENCY BASED QUESTIONS

CHAPTER-1 RECOMBINANT DNA TECHNOLOGY

1) Answer the following questions based on the information given:



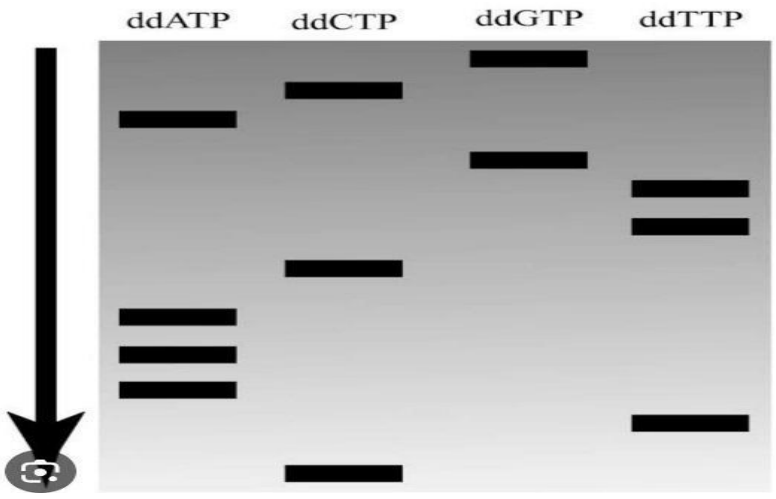
a) Identify and write the name of vector. 1

b) Can this be used as a Shuttle vector? 1

c) When the above vector is used in a RDT experiment, would the recombinant host cells survive on a culture media having antibiotic ampicillin? 1

d) In pursuing the experiment, depicted in question 'c', which method may be used for screening of true recombinants? 1

2) Observe the given autoradiogram:



a) First Read and write the sequence of original DNA. 1

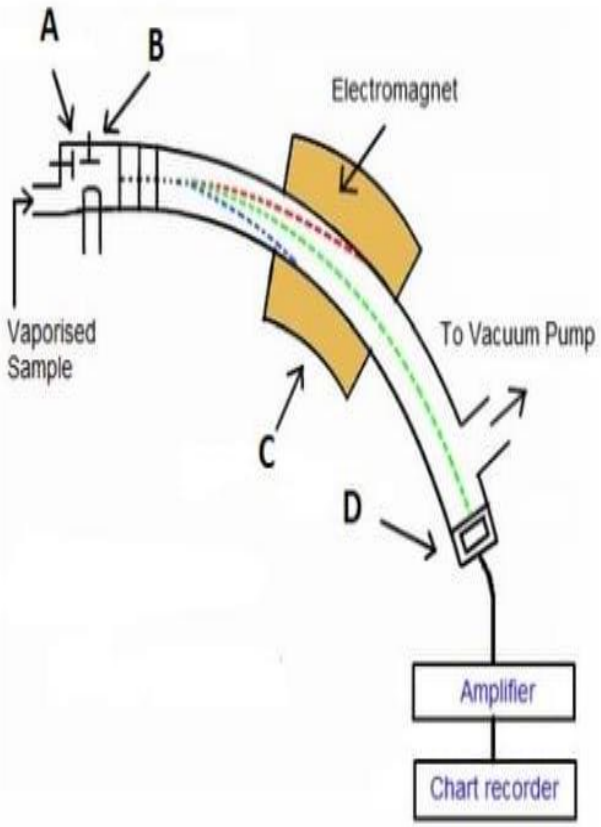
b) Name the technique used for sequencing of DNA. 1

c) Write the principle of the technique used. 1

d) Name the molecules conjugated with nucleotides used in single lane gel electrophoresis. 1

CHAPTER-2 PROTEIN STRUCTURE & ENGINEERING

1) Observe the diagram and answer the following: -

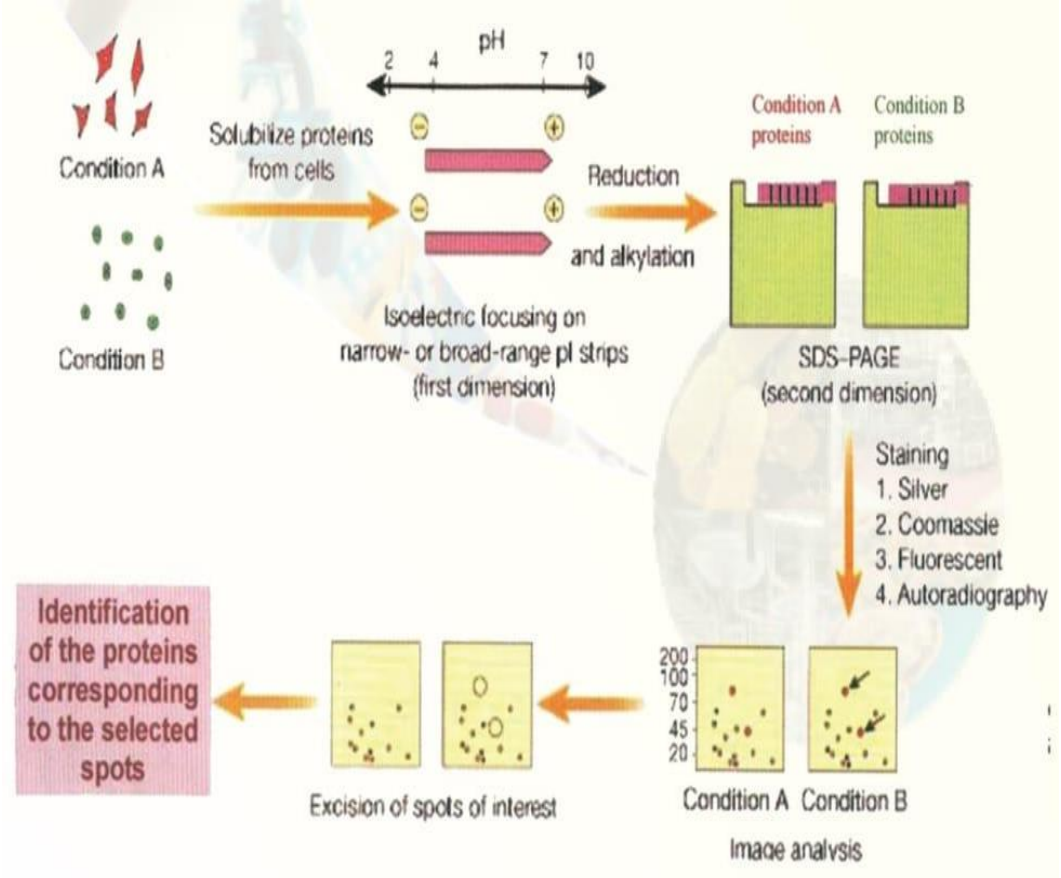


ii) Identify A, B, C and D 2

iii) Name any one technique to achieve A. 1

iv) Calculate the m/z ratio of a polypeptide that has a mass of 20 000 Da and charges +1, +2, +4, +5. 1

2) Observe the diagram and answer the questions that follow:-



a) Identify the technique depicted here in diagram. 1

b) Name the scientist who developed this technique. 1

c) White the principle used in the technique. 1

d) Name the biomolecules which are characterized through this technique. 1

CHAPTER-3 GENOMICS & BIOINFORMATICS

1) SNPs are single base positions at which different nucleotides occur within the gDNA of different individuals of a population. It has now been known that most of the sequence variation in human beings is in the form of SNPs. The variations in genetic sequences of different individuals (due to SNPs) are thought to be involved in differences in disease susceptibility, response to environmental factors, normal development and aging and drug response. The field of study that is concerned with the effect of genetic variation on drug response is known as pharmacogenomics. Pharmacogenomics is expected to develop individualized treatments that will be safer as well as more effective. Efforts are being made

to analyse the SNP pattern of those 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 of those genes and from that they can predict how the patients are likely to respond to a given drug.

Consider a situation where a most common drug for relieving the symptoms of "Asthma" namely "Albuterol" is being tested on a number of asthmetic patients. The sequences of ADRB-2 gene (controlling the production of ADRB-2 receptor on the lung cell surface which serves as the target for binding of the drug) of the patients and their response to the albuterol drug is summerised below:-

Patient's #	Response to Drug
1, 2, 5, 8, 10, 11, 12, 14	Positive
3, 4, 7, 9	No Effect
6, 13	Negative

Fig:- Patient's ADRB-2 Gene Sequences

On the basis of above data, answer the following questions (One Mark Ecah): -

a) How many SNP loci are present in the ADRB-2 gene across the patients?
b) Indicate the position of the SNP loci present within the ADRB-2 gene across the patients.
c) List out the haplotypes that are present with respect to ADRB-2 gene within the population of patients shown above?
d) An asthmatic patient, having the following ADRB-2 gene sequence, comes to see the doctor.

CTGACTAAGTACCGA

Should the doctor prescribe "Albuterol" to this patient or not? Justify your answer.

CHAPTER-4 MICROBIAL CELL CULTURE & ITS APPLICATIONS

1) Imagine you are a biotechnologist working on a project to develop a novel antibiotic using microbial cell culture techniques. You have isolated a promising strain of bacteria from a unique environmental niche. The strain shows potent antimicrobial activity against a range of pathogenic bacteria. Now, you need to optimize the microbial cell culture conditions for maximum antibiotic production.

In this context, answer the following questions: -

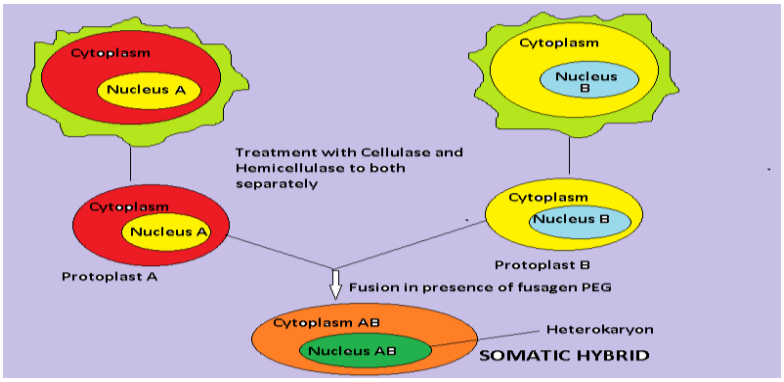
v) List out the key steps involved in initiating and maintaining a microbial cell culture for the isolated bacteria. 1

vi) Why should culture conditions be optimized during antibiotic production? 1

vii) If the concerned strain is aerobic, whether aeration and agitation will be essential in the bioreactor for maximizing the production of antibiotic at commercial level? Justify. 1

viii) What factors must be kept in mind while selecting the raw substrate to be used at commercial scale for the production of desired antibiotic? 1

CHAPTER-5 PLANT TISSUE CULTURE & ITS APPLICATIONS



a) Identify the above technique and mention one of its uses. 1

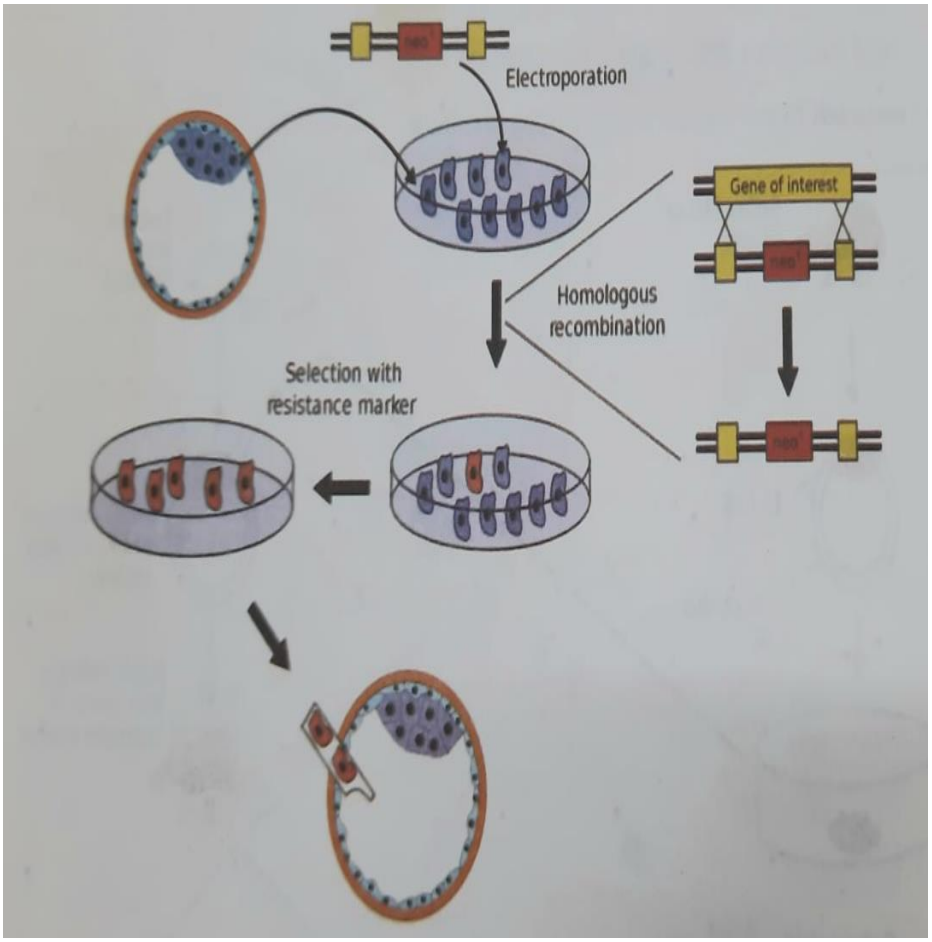
b) What are the functions of the enzymes “Cellulase” and “Hemicellulase”? 1

c) What is a protoplast? 1

d) How can one confirm the formation of somatic hybrid at molecular level? 1

e) Expand the name of the fusogenic agent “PEG”. 1

CHAPTER-6 ANIMAL CELL CULTURE & ITS APPLICATIONS



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?

e) Mention any two uses of the technique. 1

ASSERTION REASONING QUESTIONS

Question No. 1 to 35 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

CHAPTER 1: RECOMBINANT DNA TECHNOLOGY

1) Assertion: Type III restriction enzymes are used in recombinant DNA technology.
   Reason: Type III enzymes recognize 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 bacterium.

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*UC 19 is an expression vector.
   Reason: *p*UC 19 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.

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

CHAPTER 2: PROTEIN STRUCTURE & ENGINEERING

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

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

13) Assertion: Isoelectric focusing separates the proteins on the basis of their *p*I values.
Reason: At *p*I value, a protein comes to rest.

14) Assertion: The major attraction of mass spectrometer is that as little as picomoles of a protein sample can be analyzed.
Reason: Mass spectrometer analyze z/m ratio of protein ions.

15) 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.	11	12	13	14	15
ANSWER	A	B	A	C	C

CHAPTER 3: GENOMICS AND BIOINFORMATICS

16) 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.

17) 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.

18) 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.

19) 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.

20) 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.

ANSWER KEY

Q.NO.	16	17	18	19	20
ANSWER	C	D	B	A	C

CHAPTER 4: MICROBIAL CELL CULTURE & ITS APPLICATIONS

21) 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.

22) 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.

23) 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.

24) 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 favored.
Reason- Metagenomics is a powerful technique for isolation of desired culturable forms from an environmental sample.

25) 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.

ANSWER KEY

Q.NO.	21	22	23	24	25
ANSWER	A	C	B	D	A

CHAPTER 5: PLANT TISSUE CULTURE & ITS APPLIACTIONS

26) ASSERTION: Virus free plants are regenerated through apical meristem culture.
REASON: Apical meristem cells are devoid of viral infection.

27) 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.

28) ASSERTION: Auxins are added to culture medium if callus induction is desired.
REASON: Nature and quantity of auxin added depends on nature and source of explant.

29) 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.

30) ASSERTION: There are no constraints associated with public acceptance of transgenic crops.
REASON: Transgenic crops are completely safe for all forms of life and can never cause any threat to evolution and biodiversity.

ANSWER KEY

Q.NO.	26	27	28	29	30
ANSWER	A	C	B	A	D

CHAPTER 6: ANIMAL CELL CULTURE & ITS APPLIACTIONS

31) ASSERTION: Finite cell lines have limited life span.
REASON: Finite cell lines show contact inhibition and are mortal.

32) ASSERTION: To observe animal cells in culture, inverted microscopes are used.
REASON: Because media is at the bottom and the cells are at top.

33) ASSERTION: The use of blood transfusion is advantageous over EPO administration.
REASON: Blood transfusion doesn't trigger immune reactions.

34) ASSERTION: Hybridoma cells are made by fusing B cells and myeloma cells.
REASON: B cells produce monoclonal antibody and myeloma cells provide mortality.

35) ASSERTION: The ES cells are derived from the inner cell mass of growing embryos.
REASON: ICM cells show euploid karyotype.

ANSWER KEY

Q.NO.	31	32	33	34	35
ANSWER	A	C	D	A	B

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Class-XI

SUB: BIOTECHNOLOGY

MM: 70 **Time-3Hrs**

SL. NO.	UNIT	SECTION A		SECTION B	SECTION C	SECTION D	SECTION E	Total Marks
		MCQ	Assertion Based Questions	Very Short Answer Questions	Short Answer Questions	Case Based Questions	Long Answer Questions	
		12*1=12 (Q,no1-12)	4*1=4 (Q,no.13-16)	5*2=10 (Q,no 17-21)	7*3=21 (Q,no 22-28)	2*4=8 (Q,no-29-30)	3*5=15 (Q,no31-33)	70
1	BIOTECHNOLOGY AN OVER VIEW	2	0	0	1	0	0	5
2	MOLECULES OF LIFE	4	1	3	3	0	1	25
3	GENETICS AND MOLECULAR BIOLOGY	3	1	2	1	1	1	20
4	CELL AND DEVELOPMENT	3	2	0	2	1	1	20
TOTAL MARKS		12	4	10	21	8	15	70

SAMPLE PAPER 01

SUB: BIOTECHNOLOGY CLASS: XI

TIME: 3 HOURS **MM: 70**

General Instructions:

i) All questions are compulsory.
ii) Question no. 1 to 12 are MCQ based questions of one mark each.
iii) Question no. 13 to 16 are Assertion Reasoning based questions of one mark each.
iv) Question no. 17 to 21 are very short answer-based questions of two marks each.
v) Question no. 22 to 28 are short answer-based questions of three mark each.
vi) Question no. 29 to 30 are case-based questions of four mark each.
vii) Question no. 31 to 33 are long answer-based questions of five mark each.

1.	If you are researching on improving the delivery of a drug, which of the following techniques you are working on: a) Cloning b) Nanobiotechnology c) Biosensors d) Bioprocessing	1
2.	The antibiotic penicillin is a potent inhibitor of which enzyme? a) Cellulase b) DNA ligase c) Transpeptidase d) Aminoacyl tRNA synthetase	1
3.	The RNA primers formed during DNA replication are removed by: a) DNA Polymerase I b) DNA Polymerase II c) DNA Polymerase III d) DNase I	1
4.	The important event of Glycosylation occurs in: a) Lysosomes b) Golgi Complex c) Mitochondria d) Endoplasmic reticulum	1
5.	The hormones like adrenaline and thyroxine are derivatives of the amino acid: a) Serine b) Tyrosine c) Histidine d) Tryptophan	1
6.	Which of the following epithelial tissues have absorptive function? a) Squamous b) Ciliated c) Columnar d) Stratified	1
7.	Chemical mutagenesis was first reported by:	1

	a) Muller b) Stadler c) Morgan d) Auerback	
8.	Who coined the term 'Genetics'? a) Johannsen b) Bateson c) Mendel d) Tschemark	1
9.	Study of cells and their interaction with environment is called: a) Cell Biology b) Tissue Engineering c) Bioinformatics d) Microbiology	1
10.	Nobel laureate Linus Pauling contributed to the elucidation of: a) Beta Pleats b) Peptide Fingerprinting c) Alpha helix d) DNA double helix	1
11.	By nature, antibodies are: a) Lipoproteins b) Phosphoproteins c) Metalloproteins d) Glycoproteins	1
12.	Bromine water test is done for: a) Amino acids b) Saturated Fatty acids c) Unsaturated Fatty acids d) Deoxyribose sugar	1
Question no. 13 to 16 are Assertion Reasoning based questions of one mark each. ***Choose the correct option:*** a) Both assertion and reason are correct and reason is the correct explanation of the assertion. b) Both assertion and reason are correct but reason is not the correct explanation of the assertion. c) Assertion is correct but reason is false. d) Both assertion and reason are false.		
13.	Assertion: Euchromatin regions stain lightly. Reason: Euchromatin regions are less condensed and loosely coiled.	1
14.	Assertion: In Retroviruses, the genetic material is DNA. Reason: It can directly insert its genome in host.	1
15.	Assertion: After late G1, some cells withdraw from the cell cycle and enter G0 phase. Reason: These cells are metabolically inactive.	1

16.	Assertion: Genes are linked by virtue of their being located on the same chromosome. Reason: Mendel's law of independent assortment of genes is based on the independent orientation of the different homologous chromosome pairs during meiosis.	1
17.	Draw the structure of any one Sulphur containing amino acid.	2
18.	a) What will be the ratio of phenotypes expressed in F1 when a heterozygous plant is crossed with a plant that is homozygous recessive? b) What does the law of independent assortment state?	2
19.	Name and mention the roles of any two enzymes that are used in biotechnology industry.	2
20.	With the help of a cross, explain incomplete dominance.	2
21.	Name and mention in brief the strategy for sequencing of amino acids.	2
22.	Draw the following: a) dUMP b) Lactose OR Write in brief about the following tests: a) Acrolein test. b) Ninhydrin test.	1.5 X2=3
23.	What is crossing over? Mention its role in sexual reproduction.	1+2=3
24.	Explain in brief the following techniques: a) Bioprocessing b) Protein Engineering	1.5X2=3
25.	Explain any two types of genetic recombination in bacteria.	1.5X2=3
26.	a) Draw a labelled diagram of an antibody. b) What are macrophages? OR a) Draw a labelled diagram of an ovule (L.S). b) What is double fertilization.	2+1=3
27.	What properties of enzymes enable them to achieve enormously high catalytic powers?	3
28.	a) Name on protein and indicate its location for each of the following: i) Structural protein ii) Defense protein b) Name one enzyme and its coenzyme form. c) Name one form of DNA which has a left-handed helix conformation.	1X3=3

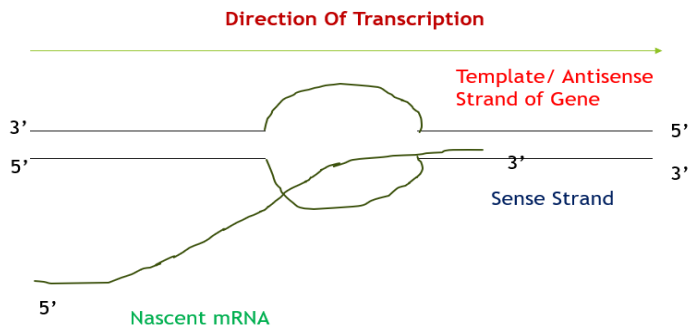
Question no. 29 to 30 are case-based questions of four mark each. Read the passages given below and answer accordingly.		
29.	In 1952, Alfred Hershey and Martha Chase took an effort to find the genetic material in organisms. Their experiments led to an unequivocal proof to DNA as genetic material. Bacteriophages (viruses that affect bacteria) were the key element for Hershey and Chase experiment. i) On which medium were the viruses cultivated by Alfred Hershey and Martha Chase? a) A medium containing radioactive potassium (K) b) A medium containing radioactive Uranium (U) c) A medium containing radioactive phosphorous (P) d) A medium containing potassium (K) ii) Which of the following is not a stage in the "Hershey-Chase experiment"? a) Blending b) Centrifugation c) Infection d) Conjugation iii) What will happen when the radioactive protein capsule of the virus is attached onto the bacteria? a) Radioactivity is detected in the supernatant b) Radioactivity is absent in the supernatant c) Radioactive DNA is injected into the bacterium d) Attachment of the virus to the bacterium doesn't occur iv) Who proved that DNA was indeed the genetic material through experiments? a) Alfred Hershey and Maclyn McCarty b) Oswald Avery and Maclyn McCarty c) Oswald Avery and Martha Chase d) Alfred Hershey and Martha Chase	1X4=4
30.	Meiosis is a mechanism in which a single cell divides twice to produce four cells that contain half of the original amount of genetic data. Those cells are our sex cells-male sperm, female eggs. One cell divides up twice during meiosis to create four daughter cells. These four daughter cells are only half as numerous as chromosomes of the parent cell-haploid. Meiosis is divisible into nine stages. These are divided between the first division of the cell (meiosis I) and the second division thereof (meiosis II). i) **The RNA and protein synthesis occurs in_____** a) M phase b)S phase c) G1 Phase d) G2 phase ii) **When does synapsis occur at Meiosis?** a) Zygotene	1X4=4

	b) Leptotene c) Diplotene d) Pachytene **iii) Cell Plate is laid during _____** a) Cytokinesis b) Karyokinesis c) Interphase d) Metaphase iv) **There are chromosomes arranged along the equator _____** a) Prophase b) Metaphase c) Anaphase d) Telophase	
31.	Explain in brief all the levels of structure of proteins.	5
32.	a) What did Meselson and Stahl observe when: i) They cultured *E.coli* in a medium containing NH4Cl for a few generations and centrifuged the content. ii) They transferred one such bacterium to the normal medium of NH4Cl and cultured for two generation. b) What did Meselson and Stahl conclude from this experiment. Explain with the help of diagram. **OR** Draw a schematic representation of the structure of the transcription unit and show the following in it. a) Direction in which transcription occur b) Polarity of two strand involved c) Template strand d) Terminator codons e) Initiation codon	2+3=5 1X5=5
33.	Identify the following organelles and write one function of each: a) form microsomes when the cell is homogenized. b) Have catalases c) Are formed from Golgi complex and contain hydrolytic enzymes d) The inner membrane is folded into cristae e) Consist of equal amounts of RNA and proteins.	1X5=5

SAMPLE PAPER- 01
SUB: BIOTECHNOLOGY CLASS: XI
MARKING SCHEME

1.	b) Nanobiotechnology	1
2.	c) Transpeptidase	1
3.	e) DNA Polymerase I	1
4.	b) Golgi Complex	1
5.	d) Tryptophan	1
6.	c) Columnar	1
7.	d) Auerback	1
8.	f) Bateson	1
9.	e) Cell Biology	1
10.	g) Alpha helix	1
11.	h) Glycoproteins	1
12.	c) Unsaturated Fatty acids	1
13.	a) Both assertion and reason are correct and reason is the correct explanation of the assertion.	1
14.	d) Both assertion and reason are false.	1
15.	c) Assertion is true but reason is false.	1
16.	b) Both assertion and reason are correct but reason is not the correct explanation of the assertion.	1
17.	Structure of Methionine or Cysteine	2
18.	a) 1:1 b) It states that alleles of two or more genes assort independently of each other during gamete formation.	2
19.	a) Restriction Endonucleases: used in RDT. b) Papain: Used as meat tenderizer. Or any other correct example.	2
20.	Organism: *Mirabillis jalapa* Trait: Flower color Parent: Red, RR (Male) X White, rr (Female) Gametes: R & r F1: Rr (All Pink flowers) Self-Cross of F1: 1 (Red, RR): 2 (Pink, Rr): 1 (White, rr)	2
21.	Explain Edman Degradation process of sequential sequencing.	2
22.	c) Correct structure OR Correct explanation of: c) Test for glycerol derived lipids with KHSO4 d) Test for amino acids with ninhydrin	

23.	Crossing over is the exchange of genetic materials during pachytene stage of Meiosis I between non-sister chromatids of homologous chromosomes. It causes variation in sexual reproduction.	1.5X2=3
24.	c) Bioprocessing: Bioprocess technology is the oldest in all the biotechnologies. Bioprocessing technology, uses living cells or the molecular components to manufacture desired products. Now a day recombinant DNA technology coupled with microbial fermentation are used to manufacture a wide range of biobased products including human insulin, the hepatitis B vaccine, the calf enzyme used in cheese making, biodegradable plastics, and laundry detergent enzymes. d) Protein Engineering: Protein engineering technology involves improvement of existing proteins, such as enzymes, antibodies and cell receptors, and to create proteins not found in nature. This technique is used in conjunction with recombinant DNA techniques. Such engineered proteins are used in drug development, food processing and industrial manufacturing.	1.5X2=3
25.	Explanation of any two of the following: Conjugation, transduction or transformation.	1.5X2=3
26.	a) Complete labelled diagram. b) Phagocytic cells derived from monocytes. Macrophages also work as antigen presenting cells (APCs) OR a) Complete labelled diagram of an ovule (L.S). b) Double fertilization is the event in which one male gamete fuses with egg that forms the zygote while the other male gamete fuses with the polar nuclei that later forms the endosperm.	2+1=3
27.	Highly specific, can work at normal atmospheric pressures and room temperature, lowers the activation energy.	3
28.	a) i) Structural protein: actin ii) Defense protein: antibody b) any correct example. c) Z DNA	1X3=3
29.	In 1952, Alfred Hershey and Martha Chase took an effort to find the genetic material in organisms. Their experiments led to an unequivocal proof to DNA as genetic material. Bacteriophages (viruses that affect bacteria) were the key element for Hershey and Chase experiment. i) c) A medium containing radioactive phosphorous (P) ii) d) Conjugation iii) a) Radioactivity is detected in the supernatant iv) d) Alfred Hershey and Martha Chase	1X4=4

30.	i) c) G1 Phase ii) a) Zygotene **iii)** a) Cytokinesis iv) b) Metaphase	1X4=4
31.	Primary structure: linear arrangement of amino acids formed by peptide bonding. Secondary structure: Local and regular arrangements due to hydrogen bonding. Forms either alpha helix or beta sheets. Tertiary Structure: High level of folding mainly due to interactions between side chains of amino acids. Quaternary Structure: Forms in proteins with more than two polypeptide chains.	5
32.	a) What did Meselson and Stahl observe when: i) Extracted DNA formed one heavy band in density gradient centrifugation. ii) Extracted DNA formed one light and one heavy band in density gradient centrifugation. iii) DNA replicates with semi conservation mode of replication. **OR**  **Start Codon** AUG; **Stop Codon**: UGA,UAG,UAA	2+3=5 1X5=5
33.	f) Endoplasmic Reticulum g) Peroxisomes h) Lysosomes i) Mitochondria j) Ribosomes	1X5=5

BLUE PRINT OF SAMPLE PAPER-1

SESSION 2023-24

Class-XII Biotechnology- (045)

MM: 70 Time-3Hrs

S. NO	UNIT	SECTION A		SECTION B	SECTION C	SECTION D	SECTION E	Total Marks
		MCQ	Assertion Based Questions	Very Short Answer Questions	Short Answer Questions	Case Based Questions	Long Answer Questions	
		12*1=12 (Q.no 1-12)	4*1=4 (Q.no. 13-16)	5*2=10 (Q.no 17-21)	7*3=21 (Q.no 22-28)	2*4=8 (Q.no-29-30)	3*5=15 (Q.no 31-33)	70
1	RECOMBINANT DNA TECHNOLOGY	3	1		2		1	15
2	PROTEIN STRUCTURE & ENGINEERING	3	1	2	1	1		15
3	GENOMICS & BIOINFORMATICS	2		1	2			10
4	MICROBIAL CELL CULTURE & ITS APPLICATIONS	2	1	2		1		11
5	PLANT TISSUE CULTURE & ITS APPLICATIONS	1	1		1		1	10
6	ANIMAL CELL CULTURE & ITS APPLICATIONS	1			1		1	9
TOTAL MARKS		12	4	10	21	8	15	70

SAMPLE PAPER-01
BIOTECHNOLOGY
Class- XII

General Instructions:

i) All questions are compulsory.

ii) The question paper has five sections.

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 is a palindrome?
   a. GAATTC
   b. ATTCGG
   c. ATGCGC
   d. GCTTGG
2. Which of the following REs will produce a blunt end?
   a. *Eco*RI
   b. *Bam*H*I*
   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 which grows at temperature 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 of 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 there is a need of 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?

OR

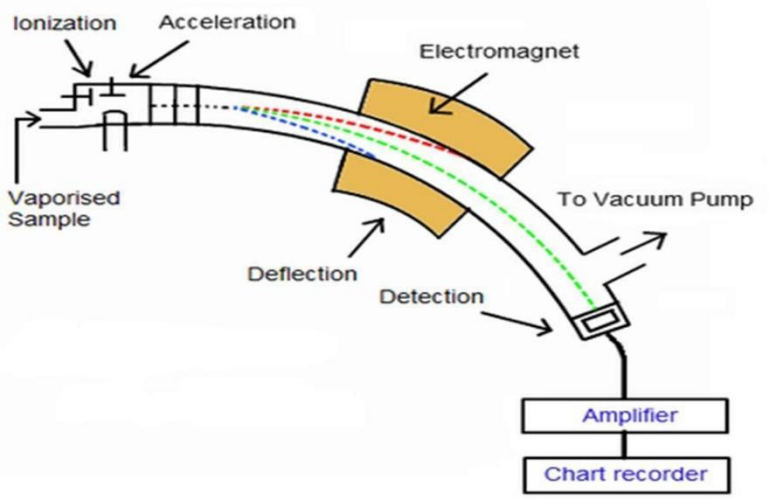
How is FISH used to study the effectiveness of anti-cancer drug on CML patients? Draw diagram to show reciprocal translocation. [3]

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. In which form, sample has to be introduced in mass spectrometer?

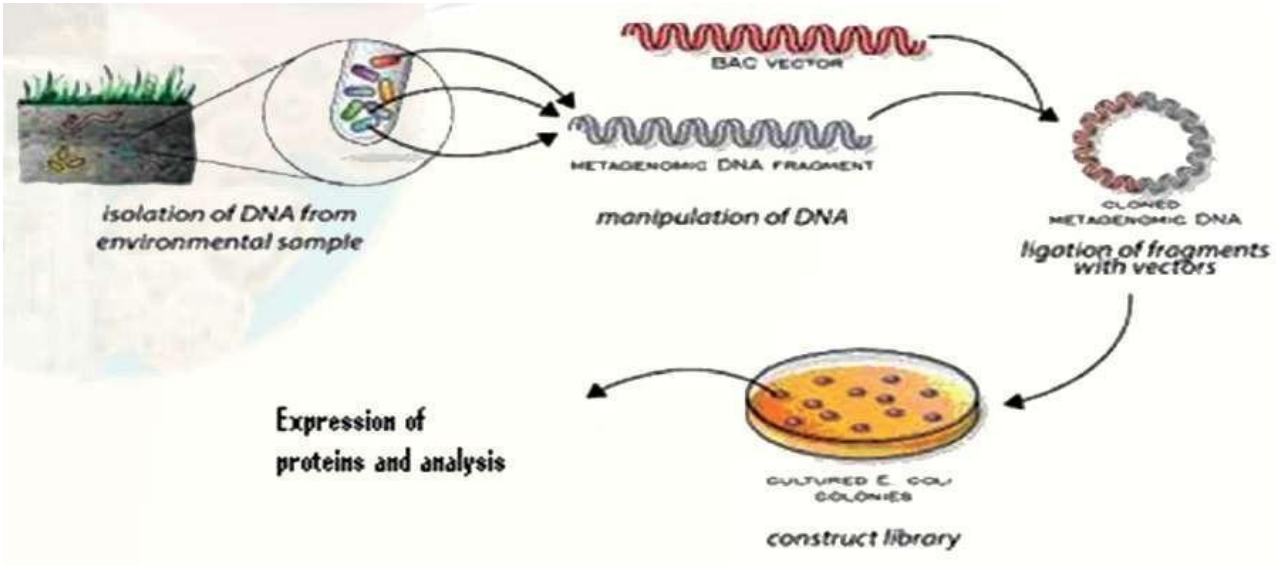
b. What is the advantage of mass spectrometry?

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. What 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.

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 to produce golden rice? How golden rice is 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) Give the example of GM Crop with the following resistance along with the name of gene(s) used for their genetic modification-

- *Helicoverpa armigera* resistant
- 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 *p*UC 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 *p*UC? [1+2+2]

SUGGESTIVE MARKING KEY
SAMPLE PAPER-1 - BIOTECHNOLOGY –CLASS-12th
EXPECTED ANSWERS/VALUE POINTS

SECTION-A

1) a GAATTC 1

2) d *Alu*I 1

3) a Plasmid< λ-phage < Cosmid < BAC <YAC 1

4) c Purification of protein, trypsin treatment, paper electrophoresis, paper chromatography 1

5) d These compounds increase the rate of reaction of acidic serine residue 1

6) a Whey, milk, casein, soya, rice, wheat 1

7) a Deletion of 3bps resulting in loss of codon 508 1

8) b Sequencing the whole genome 1

9) b *Aspergillus oryzae* – Vitamin B12 1

10) a Explant, sterilization, transfer to medium, incubation, organogenesis, hardening 1

11) d It is designed to maintain high CO concentration with O_2 concentration 1

12) c Provide microbial growth medium and incubate at 50^{O} C 1

13) b Both Assertion and Reason are true but the reason is not the correct explanation of the assertion 1

14) a Both Assertion and Reason are true and the reason is the correct explanation of the assertion 1

15) a Both Assertion and Reason are true and the reason is the correct explanation of the assertion 1

16) a Both Assertion and Reason are true and the reason is the correct explanation of the assertion 1

SECTION-B

17) Direct production of microbes on a large or commercial scale has the risk of not only large investments, but also producing products, which may not be of appropriate quality 2

18) Whey proteins result in the elevation of a tripeptide 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. 2

OR

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 or athletes who want to protect their existing mass, the idea is to take BCAA enriched foods before and after exercise. 2

19) Aeration- To achieve uniform oxygen concentration.
Agar- Solidifying Agent
Antifoams- Prevention of foaming
Corn-steep Liquor- Nitrogen Source at commercial scale. 2

20) 2D- Electrophoresis: Separation of different proteins/peptide fragments in two dimensions using two different techniques/parameter 1
It is better because it provides better resolution. 1

21) a- Protein Data Bank, b- UniGene, c- RefSeq, d- GenBank 2

SECTION-C

22) Single nucleotide polymorphisms, frequently called SNPs (pronounced "snips"), are the most common type of genetic variation among people. Each SNP represents a difference in a single DNA building block, called a nucleotide. For example, a SNP may replace the nucleotide cytosine (C) with the nucleotide thymine (T) in a certain stretch of DNA.
SNPs occur normally throughout a person's DNA. They occur almost once in every 1,000 nucleotides on average, which means there are roughly 4 to 5 million SNPs in a person's genome. These variations occur in many individuals; to be classified as a SNP, a variant is found in at least 1 percent of the population. Scientists have found more than 600 million SNPs in populations around the world.
Most commonly, SNPs are found in the DNA between genes. They can act as biological markers, helping scientists locate genes that are associated with disease. When SNPs occur within a gene or in a regulatory region near a gene, they may play a more direct role in disease by affecting the gene's function.
Most SNPs have no effect on health or development. Some of these genetic differences, however, have proven to be very important in the study of human health. SNPs help predict an individual's response to certain drugs, susceptibility to environmental factors such as toxins, and risk of developing diseases. SNPs can also be used to track the inheritance of disease-associated genetic variants within families. Research is ongoing to identify SNPs associated with complex diseases such as heart disease, diabetes, and cancer. 3

23) 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.

3

24) Cells are dissociated from the parental tissue (such as kidney, liver) by mechanical or enzymatic methods and maintained in suitable culture medium and vessels. The most frequently used enzymes for separating cells from a given tissue (dispersion) are crude.

25) preparations of trypsin and collagenase that cleave the proteinaceous cementing material between cells in a tissue. The maintenance of growth of such cells under laboratory conditions is known as primary cell culture.

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. 3

26) Differences between primary and secondary metabolites

Primary metabolite- Amino acid, nucleotide, fatty acids, etc.

Secondary Metabolite- Antibiotics 3

27) dNTPs have –OH group at 3’ carbon but ddNTPs do not.

5’-TA-3’ and 5’-TACGCCA-3 3

Two primers are needed for copying both strands of DNA.

Thermostable DNA polymerase is needed to carry out polymerization of DNA at elevated temperature, i.e., above 70 ^{0} Celsius.

$5*2^{25}$ molecules will be formed. 3

BLAST- Basic Local Alignment Search Tool- Sequence similarity search tool helping to detect homologous sequences.

28) BLAST search enables a researcher to compare a subject protein or nucleotide sequence (called a query) with a library or database of sequences, and identify database sequences that resemble alphabet above a certain threshold. For example, following the discovery of a previously unknown gene in the mouse, a scientist will typically perform a BLAST search of the human genome to see if humans carry a similar gene; BLAST will identify sequences in the human genome that resemble the mouse gene based on similarity of sequence. 3

OR

The application of FISH can be illustrated by taking an example of chronic mylogenous leukemia (CML). It was observed from the karyotype analysis of the lymphocyte preparation made from blood samples of CML patients that there was a 9-22 translocation in the chromosome (also called 'Philadelphia chromosome'). Although by counting the number of such cells it was possible to find out the severity of the disease, it was not an easy procedure. The regions on the chromosomes involved in

translocation were identified on chromosomes 9 and 22. From the DNA library it was possible to pick up clones carrying the particular genes involved in CML. Using nick translation it was possible to fluorescently label chromosome 9 region with red colour and chromosome 22 region with green colour and prepare the probe. It was observed that when CML lymphocytes smear cells were hybridized with the two probes in situ and when observed under fluorescent microscope, the cells, which were affected, appeared yellow (mixing of green and red colour produces yellow colour). The unaffected cells appeared as red and green. This technique known as Fluorescence in situ Hybridization (FISH) allows knowing the status in the interphase unlike in karyotyping where you need a metaphase chromosome. The status of the disease could easily be identified by counting the number of cells, which appeared yellow . Further, it was possible to monitor the effect of chemotherapy and drugs by taking out samples and counting the number of cells appearing yellow.

3

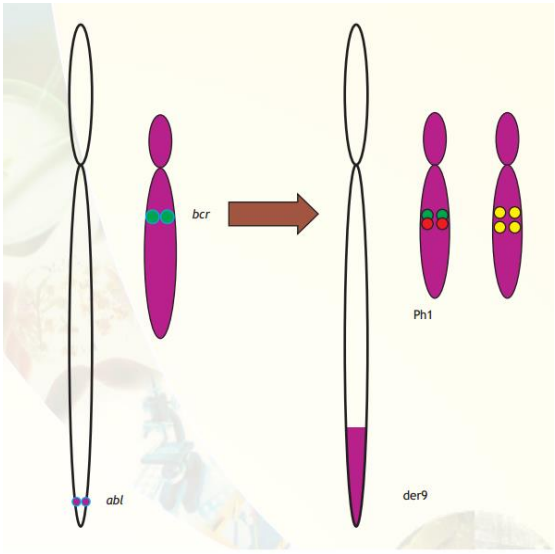


Diagram- Reciprocal Translocation

SECTION-D

29) a. Vaporized form

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

c. A mass spectrometer (or mass spectrograph) consists of three essential parts: the ionization chamber, the deflection chamber, and the detector. The ionization chamber is a region in which atoms of the unknown material are excited so as to make them lose electrons.

Sometimes the energy needed for exciting the atoms is obtained simply by heating the sample. When atoms are excited, they lose electrons and become positively charged particles known as ions.

OR

50001, 25001, 16667.66, 12501 1+1+2

30) a. Metagenome is the recovery and complete sequencing of genetic material extracted directly from all environmental samples.

b. Metagenome encompasses both culturable and un-culturable forms.

c. We can isolate the gene using metagenomics approach and clone the gene followed by its expression in the suitable host so as to purify the antibiotic using at commercial scale.

OR

Genomic DNA

↓

Restriction Digestion

↓

Insertion of Restriction Fragments into vector to form rDNA

↓

Transformation of rDNA into host and selection

↓

To grow transformed cells in-vitro so as to get the library.

1+1+2

SECTION-E

31) Stem cells found in Inner Cell Mass of blastocyst stage of embryo.
They are obtained from ICM of blastocyst stage of embryo.
They are maintained by animal cell culture in-vitro.
Any two properties of Embryonic Stem Cells 5

OR

Method to do secondary cell culture
Flowchart of the steps of the method
Method of Establishment of cell lines 5

32) (i) Golden rice a transgenic crop rich in provitamin-A. It was created by genetic modification of rice genome with the gene coding for provitamin-A.

(ii) By using seed specific promoter

(iii) Bt-cotton having cry1Ac gene,
Virus resistant tobacco plant variety having virus coat protein gene(s) 5

OR

Micropropagation- In-vitro clonal multiplication of elite plant species using plant tissue culture methods.
It's not seasonal, labor-intensive and time consuming.
Steps of Micropropagation
Any two advantages of micropropagation 5

33) (a) We need selection because we need only host cells transformed with recombinant DNA from among the cells transformed with unaltered vector and non-transformed ones.

(b) Blue-White Colony Selection Method

(c) Insertional inactivation- Inactivation of a gene by insertion of a DNA fragment within it is known as insertional inactivation. 5

OR

(a) Ori- Origin of replication

(b) MCS provided flexibility in the choice of restriction enzyme to be used to cut the vector.

*p*UC19, marker gene- lacz'

Shuttle vector can work in two different host species, *p*UC19 can work only in *Escherichia coli*

SAMPLE PAPER- 02
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 2 case based question of **4 marks**; **Section-E** has 3 long answer questions of **5 marks** each.
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	Male sterility is widely used in crops such as maize, sunflower for hybrid production. Male sterile plants are created by introducing a gene encoding- (a) Barnase protein (b) TA29 (c) Barstar protein (d) Coat protein	1
2	Body builders prefer to drink buffalo milk to build muscle mass. Determine the reason for this? (a) Easier to digest (b) Lower fat content (c) Higher calcium and phosphorus content (d) Balanced calorie source	1
3	An industrially important secondary metabolite which is used as a red pigment in lipstics and dye for silk is obtained from- (a) *Datura stramonium* (b) *Lithospermum erythrorhizon*	1

	(c) *Digitalis lanata* (d) *Coptis japonica*	
4	Proteome of a given cell is dynamic because : (a) In response to Internal and external changes the biochemical machinery of the cell could be changed. (b) In response to Internal and external changes the biochemical machinery of the cell could not be changed. (c) No direct relationship exists between Internal and external changes in the biochemical machinery of the cell. (d) Indirect relationship exists between Internal and external in changes the biochemical machinery of the cell.	1
5	Artificial seeds are produced by- (a) Encapsulating somatic embryos in calcium alginate beads (b) Desiccating the somatic embryos with or without coating (c) Hydrating the somatic embryos (d) Hydrating the zygotic embryos.	1
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: (a) Plant tissue culture (b) Adding fertilizers to soil (c) Protein engineering (d) Vegetative Propagation	1
7	From Blue-White selection, we infer that: (a) White colonies represent non-recombinant bacteria	1

	(b) Blue colonies represent non-recombinant bacteria (c) Blue colonies represent recombinant bacteria (d) Blue and white colonies represent non-recombinant bacteria	
8	A piece of young hypocotyl was cultured in MS medium in a plant tissue culture lab. This is a type of- (a) Organ culture (b) Callus culture (c) Explant culture (d) Mass cell culture	1
9	Molecular Biologists prefer to use artificial vectors with MCS. List a benefit for this choice. (a) Flexibility in choice of insert size (b) Flexibility in choice of vector size (c) Flexibility in choice of host organism (d) Flexibility in choice of restriction enzyme	1
10	Native enzyme Subtilisin is inactivated by bleach, in detergents because of oxidation of methionine at position 222 (Met222). Choose a strategy that will help overcome this problem: (a) Use Pepsin instead of Subtilisin (b) Eliminate use of bleach (c) Substitute another amino acid at position 222 (d) Use Amylase instead of Subtilisin	1
11	Culture based approaches for detecting pathogens, as compared to PCR based assays are – (a) Faster, safer but less specific	1

	(b) Faster, safer and more specific (c) Slower, less safe and less specific (d) Slower, less safe but more specific	
12	A 100 Kb DNA fragment has to be cloned in a host cell. Which vector should be used for this experiment? (a) Plasmid (b) Cosmid (c) BAC (d) Bacteriophage lambda	1
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**-The functional property of whey protein exploited in confectionery is browning. **Reason**-Whey proteins undergo Maillard reaction providing color and aroma to food items	1
14	**Assertion-** Foaming is a problem in most microbiological processes. **Reason**- It is caused due to the presence of fatty acids and silicones in the culture medium.	1
15	**Assertion**- Whey mixed with herbs and honey is administered to the sick to treat ailments like jaundice and infected skin lesions.	1

<table>
<tr><td></td><td>Reason - Whey proteins elevates the levels of glutathione which protects the cells from harmful oxygen intermediates.</td><td></td></tr>
<tr><td>16</td><td>Assertion-It’s difficult to count genes even if we know where the genes are in a given genome

Reason- There is no simple correlation between the intuitive complexity of an organism and the number of genes in its genome.</td><td>1</td></tr>
<tr><td colspan="3">SECTION B</td></tr>
<tr><td>17</td><td>Depict the production and mode of action of tissue plasminogen activator through diagram or flowchart.</td><td>2</td></tr>
<tr><td>18</td><td>X is a valuable tool in plant breeding, wherein variation in tissue culture regenerated plants from somatic cells can be used for the development of crops with novel traits. Identify "X". State any one example where this tool can be used for crop improvement.

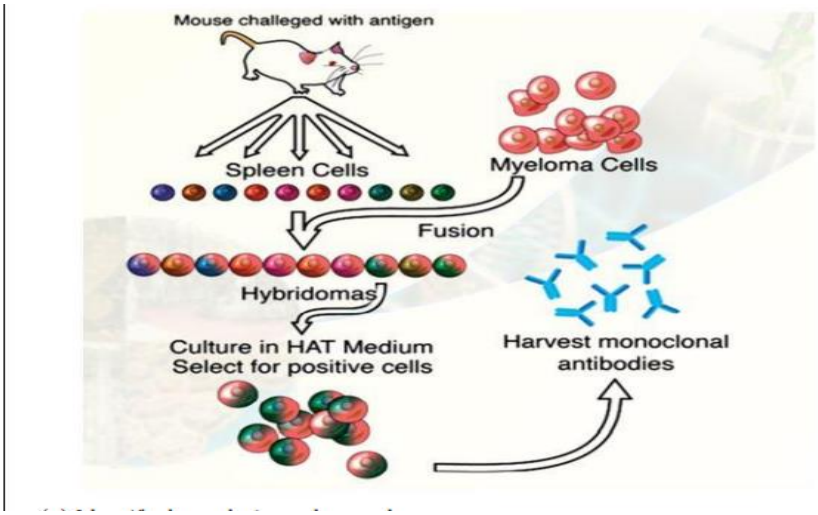
OR

Leaf explants of brinjal are showing multiple shoot regeneration in a plant tissue culture laboratory. Which plant regeneration pathway is depicted here? In this process, what would happen if either auxins or cytokinins are high in the medium?</td><td>1+1=2</td></tr>
<tr><td>19</td><td>Given below is a list of the first 06 residues of the beta helix in myogloblin from different organisms. Based on this information, which amino acids

(a) are most conserved, and

(b) are highly variable.

<table>
<tr><td>Position →
Organism↓</td><td>1</td><td>2</td><td>3</td><td>4</td><td>5</td><td>6</td></tr>
<tr><td>Human</td><td>D</td><td>I</td><td>P</td><td>G</td><td>H</td><td>G</td></tr>
<tr><td>Chicken</td><td>D</td><td>I</td><td>A</td><td>G</td><td>H</td><td>G</td></tr>
</table>
</td><td>1+1=2</td></tr>
</table>

	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	
20	A doctor has to prescribe a protein rich diet to sportsmen to improve their performance. What are the two parameters that the doctor should consider while prescribing these protein sources. Explain.							1+1=2
21	Observe the given picture and answer followings:  (a) Identify the technique shown above. (b) State any three applications of the technique.							2
	SECTION C							
22	(a) Chymotrypsinogen is inactive form of enzyme chymotrypsin. Which molecular alteration converts it into active form? (b)The catalytic triad in chymotrypsin leads to a charge relay system. Justify.							1+2=3

<table>
<tr><td></td><td>OR

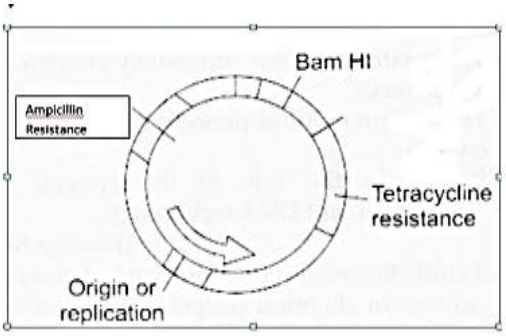
Hemoglobin protein of a normal individual has to be compared with that of a person with sickle cell anemia in a pathology laboratory. Represent the steps of the technique, which can be used for the same, in the form of a flow chart.</td><td>3</td></tr>
<tr><td>23</td><td>Given below are few transgenic crops approved by US Food and Drug Administration along with the improved character. Name the genes A to F introduced for the improved character.

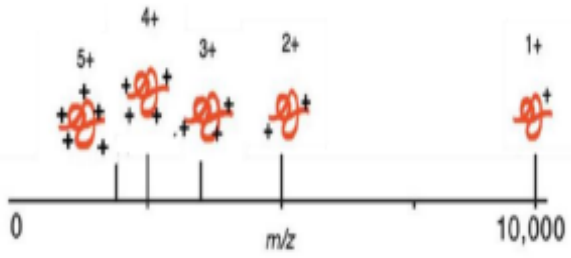
<table>
<tr><th>Crop</th><th>Gene</th><th>Improved character</th></tr>
<tr><td>Canola l</td><td>A</td><td>Hybrid production</td></tr>
<tr><td>Corn</td><td>B</td><td>Insect resistance</td></tr>
<tr><td>Cotton</td><td>C</td><td>Insect resistance</td></tr>
<tr><td>Papaya</td><td>D</td><td>Virus resistance</td></tr>
<tr><td>Potato</td><td>E</td><td>Insect and virus control</td></tr>
<tr><td>Soyabean</td><td>F</td><td>Weed control</td></tr>
</table></td><td>3</td></tr>
<tr><td>24</td><td>In animal cell culture, osmolarity of the culture medium has significant role in cell growth and function. Justify. Which ingredients decide osmolarity of the medium.</td><td>3</td></tr>
<tr><td>25</td><td>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) Belongs to the chymotrypsin family

(c) Has a database that can we used to trace the evolutionary history of this proteolytic protein.</td><td>3</td></tr>
</table>

26	What are type II restriction endonucleases (RE)? Give an example of a type II RE that generates flush ends and the sequence recognized by it. Mention two other enzymes and their utility in cloning experiment.	3
27	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.	3
28	 a) Identify the vector shown in figure. b) How can we use *LEU2* gene as a selectable marker?	3
	SECTION D	
29	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 charge, respectively, as shown below.	1+1+2=4



(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?

30

Growth kinetics is an autocatalytic reaction which implies that the rate of growth is directly proportional to the concentration of cell. As the cell divides, we shall have-

No. of cell division	0	1	2	3	n
No. of cells	1	2	4	8	2^0
Mathematically	N_0	$N_0 \times 2^2$	$N_0 \times 2^2$	$N_0 \times 2^2$	$N_0 \times 2^2$

Doubling time which is the time taken by the population to double through one round of cell division is inversely related to specific growth rate.

(a) In a microbiology laboratory, one bacterial culture is marked "X" with generation time 20 s and other bacterial culture is marked "Y" with generation time 30 s. Which bacterial culture will proliferate rapidly?

(b) Using the above table, Calculate the number of divisions the population must have undergone to increase from 104 to 107 in 24 hours.

1+1+2=4

	(c) Using the above table , Calculate the generation time (doubling time) of a bacterial population in which the number of bacteria increases from 108 cells/ml to 1014 cells/ml during four hours of exponential growth. **OR** (c) Explain any two different ways to measure microbial growth.	
	SECTION E	
31	Several medically important protein pharmaceuticals have been produced using animal cell culture and recombinant DNA technology. Represent the animal cell line used for the production of the following proteins and their therapeutic use in a tabular form. (a) Erythropoietin (b) Factor VIII (c) Follicle Stimulating Hormone (FSH) (d) Interleukin 2 (IL 2) (e) Monoclonal antibodies (mAbs) **OR** (a) Differentiate between- (i) Defined and Serum-supplemented medium (ii) Anchorage-dependent and Anchorage-independent cells (b) Explain how pH is maintained in animal cell cultures. Mention two advantages of maintaining pH during such cultures.	5
32	a) Dr. Sharma discovered first restriction enzyme ever from a bacteria called *Thermus aquaticus*, strain DR 15. Name the enzyme. b) Design two primers (5 nucleotide long each) for the given sequence:	5

	5'GATTCATTGCGCGCATTACTCGCATT 3' c) Recognition sites are generally palindromic in nature. Does it point towards the structure of restriction enzymes being that of a homodimer or heterodimer? Give reason for your answer. d) A bacteriophage is known to infect *E.coli* with pili. How can it be modified to serve as a suitable vector?	
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. **OR** a) A professor told her students to be ready a bacterial culture in 12 hours sharp. Suggest her students two ways to enhance the growth of bacterial cells in the lab so that they are able to fulfill the requirement. b) Write any two commercial significance of microbial cell culture. c) There are many ways of measuring microbial growth. Which technique is considered the best and why?	5

SAMPLE QESTION PAPER
SET -2
Marking Scheme
BIOTECHNOLOGY (045)
Class-XII

SECTION-A		
1	(a) Barnase protein	1
2	(c) Higher calcium and phosphorus content	1
3	(b) *Lithospermum erythrorhizon*	1
4	(a) In response to Internal and external changes the biochemical machinery of the cell could be changed.	1
5	(a) Encapsulating somatic embryos in calcium alginate beads	1
6	(c) Protein engineering	1
7	(b) blue colonies represent non recombinant bacteria	1
8	(c) Explant culture	1
9	(d) Flexibility in choice of restriction enzyme	1
10	(c) Substitute another amino acid at position 222	1
11	(c) Slower, less safer and less specific	1
12	(c) BAC	1
13	A) Both Assertion and Reason are true and the reason is the correct explanation of the assertion	1
14	(C) Assertion is true but Reason is false	1
15	(A) Both Assertion and reason are true and reason is the correct answer for the assertion.	1

16	A) Both Assertion and Reason are true but the reason is not the correct explanation of the assertion	1

SECTION-B

17	Tissue Plasminogen Activator (tPA) Diagram /Flow chart (Either one)	2

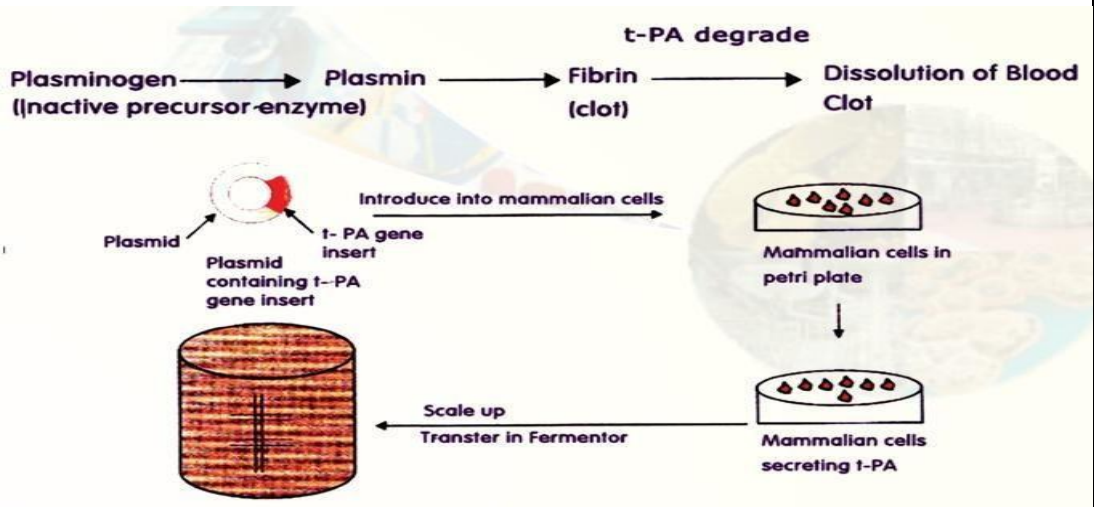
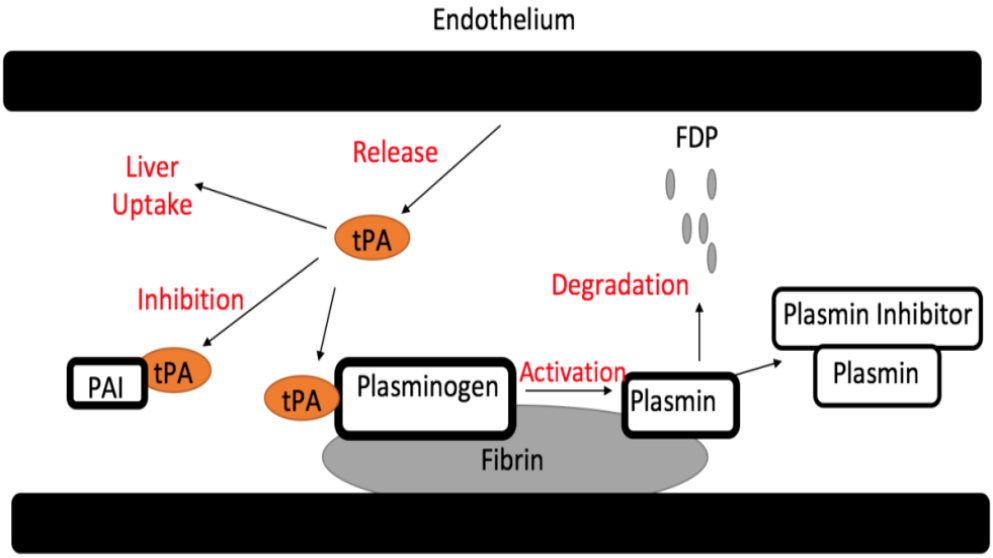


Fig. 6. Production and mode of action of tPA.

Flow chart



18	Somaclonal variations It helps in production of mutants e.g. disease resistance in Potato **OR** Organogenesis If auxins are high in the medium, it promotes rooting while if cytokinins are high, shoot formation is promoted.	1+1
19	G amino acid is most conserved A amino acid is most variable.	1 1
20	**Essential amino acids and BCAA profile**: Essential amino acids are those amino acids which have to be obtained from food and cannot be made in our cells. 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. **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 is arranged in decreasing order- whey, milk, casein, soya, rice, wheat. (Any two)	1 1
21	a) Production of MoAb (0.5 mark) b) This technology has revolutionized the area of diagnostics and antibody-based therapies. 1) The availability of monoclonal antibodies has helped in the early detection of many infectious diseases like hepatitis and AIDS. 2) Therapeutic mAb – **OKT3** Therapeutic mAb - Herceptin OKT-3 is monab-CD3, an immunosuppressant drug given intravenously to reverse the acute rejection of transplanted organs such as the heart, kidney and liver. **Herceptin (trastuzumab)** is a monoclonal antibody approved for therapy of early-stage breast cancer that is Human Epidermal growth factor Receptor 2-positive (HER2+). (1.5 marks)	1 + (0.5*3=1.5) = 2

SECTION-C

22

(a) In chymotrypsinogen, the substrate binding site is blocked and hence the enzyme is inactive. In-situ activation of trypsin involves a proteolytic cut in chymotrypsinogen which results in a conformational change, exposing the substrate binding pocket.

(b) Asp 102, His 57 and Ser 195 lie in this order forming a charge relay;

The negatively charged aspartate carboxylate residue pulls the Ser –OH proton through His, leaving it with a negative charge Ser195 becomes acidic due to the unique constellation of the three amino acid residues because the protein has folded uniquely in space

1+2

O
R

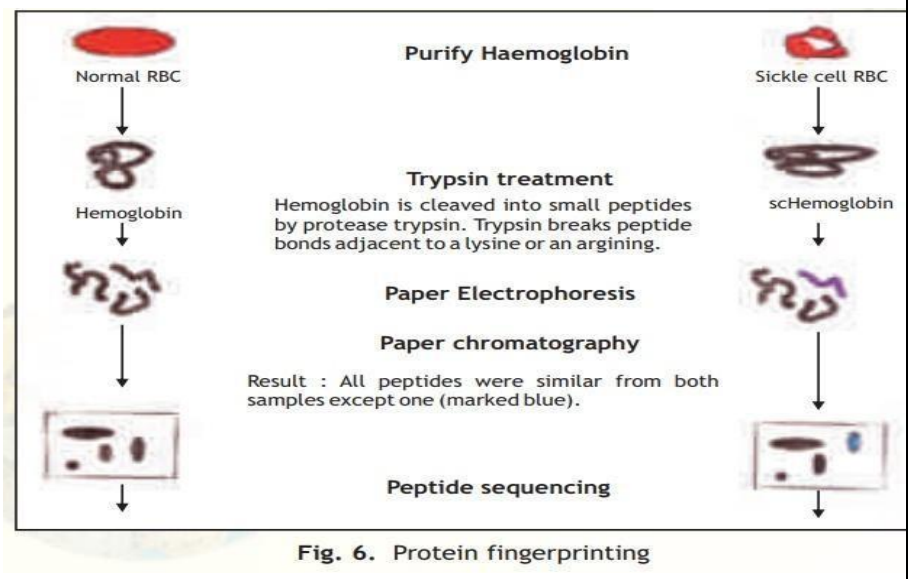


Fig. 6. Protein fingerprinting

Protein fingerprinting/ peptide mapping

½ x 6

23

Crop	Gene	Improved Character
Canola	(A) Barnase Barstear	Hybrid production
Corn	(B) BtCrylA(c)	Insect Resistance
Cotton	(C) BtCrylA(c)	Insect Resistance
Papaya	(D) Coat protein	Virus Resistance
Potato	(E) BtCrylllA & Coat protein	Insect & virus control
Soyabean	EPSP synthase	Weed control

½ x 6

24	Membrane integrity maintained Helps to maintain the shape and size of cells. Salt, glucose and amino acids (any two) are the major ingredients that determine osmolality of the medium.	1x 3
25	(a) →BLAST search→ Find out→ homologous sequences in other organisms by looking for gene sequence of given proteolytic enzyme. (b) Look for conserved domain and find whether belongs to domain of Chymotrypsin or to other family of proteins (c) ALI database can be used for Phylogenetic (Evolutionary) analysis and alignment of proteins.	1 1 1
26	R.E. type II recognizes a specific DNA sequence and cut within the sequence generating sticky/flush ends. In recombinant DNA technology, we use type II RE as they are highly specific in their action. Alu I with the restriction site (One strand) 5‟ AGCT‟3 and Sma 1 with the restriction site 5 „CCC GGG‟ 3 (flush ends) (One strand) The functions of a) Alkaline phosphatase b) DNA ligase. *The role of alkaline phosphatase is to prevent self re-ligation of the vector *The role of DNA ligase is to make 3‟-5‟ phosphodiester bond.	1 1 ½ ½
27	*i) UniGene database* *ii) Homologene database* *iii) RefSeq database*	1 1 1
28	*a) p BR 322*	1
	b) LEU2 gene codes for an enzyme required for the synthesis of amino acid leucine. Yeast cells having this plasmid can grow on a medium lacking leucine and hence can be selected e.g. Yep	1 ½ ½

SECTION- D

29	(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) (c) (c)m/z= $(M+ nH)^{n+}/ n^{+}$ 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** (c) m/z= $(M+ nH)^{n+}/ n^{+}$ For n=6, m/z= 20,000+ 6/6= 3334.33 For n=7, m/z= 20,000+7/7=2858.14	1 1 2
30	a) As generation time is inversely related to specific growth rate, hence bacterial culture marked "X" with generation time 20s will **proliferate rapidly.** b) n = 3.3 (Log 10^7 – Log 10^4) = 3.3 (7 – 4) = 10 c) First calculate the number of divisions the population must have undergone to increase from 10^8 to 10^{14} in 4 hours. n = 3.3 (Log 10^{14} – Log 10^8) = 3.3 (6) = 19.8 t_d = 240 minutes / 20 = 12 minutes **OR** c) (i) Measurement of Dry mass and Wet mass (ii) Using spectrophotometer (iii) Using Slide counting Chamber (iv) Using Coulter chamber (*Any two*)	1 1 2

SECTION- E

31

Proteins	Animal cell line used	Therapeutic use
Erythropoietin	CHO cells	Anemia
Factor VIII	CHO cells	Hemophilia A
Follicle Stimulating Hormone (FSH)	CHO cells	Infertility
Interleukin 2 (IL 2)	CHO cells	Cancer therapy
Monoclonal antibodies (mAbs)	Hybridoma cells	Cancer therapy & Autoimmune diseases

½ x10

2

OR

(a) (i) A defined medium has known chemicals, of fixed composition and can support growth of selected cells.Serum is an essential component of animal cell culture media and is a source of growth factors and hormones.

(ii) Anchorage dependent cells grow as adherent cells whereas anchorage-independent cells grow as suspension cultures. 2

(b) Most common buffering system used to maintain pH in animal cell Culture is Bicarbonate-CO_2system.Carbon dioxide from cells or atmosphere interacts with water and leads to drop in pH.

$$H_2O + CO_2 \text{《----》} \underset{HCO_3^{-1}}{H_2CO_3} \text{《-----》} (H^{+})^{+}$$

Increase in Bicarbonate concentration neutralizes the effect of increased Carbon dioxide according to the following equation: 1

$$NaHCo_3 \text{ ---> } Na^{+} + HCO_3^{-1}$$

The increased HCO3- ions derive the above equation to its left until equilibrium is reached at pH 7.4

Advantages :

i) pH is important to maintain in balance/ enzyme functions/ binding of hormones/growth factors to cell surface receptors/Ion balance (Any two)

32

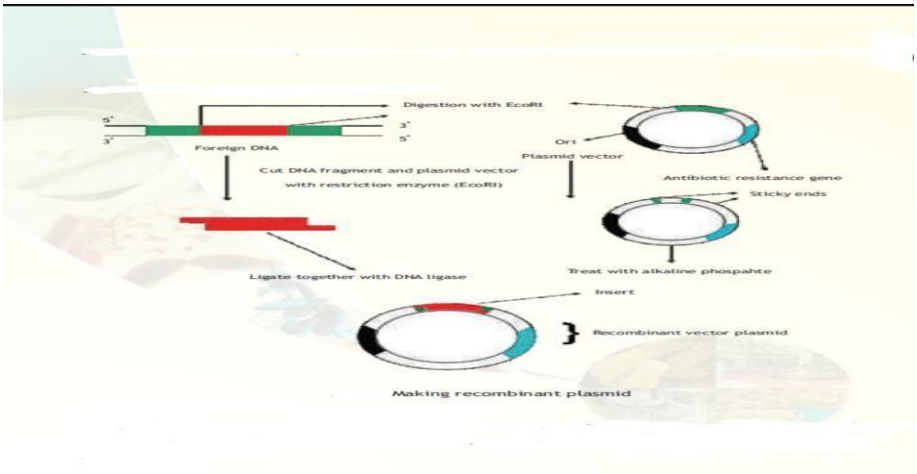
(a) TaqDI 1

(b) 5' AATGC 3' and 5' GATTC 3' 1

(c) Palindromic means the DNA sequence reads same when read from 5' to 3'. The Restriction enzyme is a homodimer. ½

As it cuts both the strands of DNA simultaneously in 5' to 3' direction.

(d) Foreign DNA can be inserted into bacteriophage single stranded, circular ½

		DNA of 6407 bp without disrupting any of the essential genes 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).	2
		OR **a)**  **b) Replica plating**. ● Host cells are first plated (master plate) on solid media with the desired antibiotic overnight. ● Velvet paper is aligned, pressed on master plate. ● With the same alignment it is pressed onto the replica plate. ● Keep it overnight, transformed colonies will not grow in replica plate ● The colonies having insert can easily be scored off from master plate by comparing the two plates.	2 ½ ½ *5
	33	(a) Recombinant insulin is an intracellular protein so we need to process the cell mass and not the fermentation broth. (b) Strain improvement is done in order to maximize metabolite production by: i) Mutant selection: There are two methods - Physical method & Chemical Method ii) Genetic engineering (c) i) It has strong inducible promoters ii) It is capable of making post-translational modifications similar to those performed by human cells iii) Downstream processing is simpler as Pichia does not secrete its own proteins into the fermentation medium. **(*Any two*)** **OR**	1 1 1 1 x 2

	a) <u>Use of shake culture and Use of baffle flask</u> Baffle flask: One of the simplest ways is to produce a V- shaped notch or indentation in the sides of the flask. Such flasks are called baffle flasks. This improves the growth of the microbes by improving the efficiency of oxygen transfer due to increased turbulence of the agitated culture medium. Shakers: Continuous agitation of the culture medium also greatly improves the efficiency of the oxygen transfer and this improves the growth of the microbes. In the laboratory, this is done by the use of shakers. Shakers may be end-to-end type or rotatory type. These may be designed for use at the ambient temperature or in a controlled temperature environment (incubator shaker). b) 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) Bioremediation/fermented food items/ recombinant proteins (Any two) **c)** Viable Plate Count is the best method since it does not count dead microbial cells.	1x2 1x2 1

BLUE PRINT OF SAMPLE PAPER-2
SESSION 2023-24
Class-XII Biotechnology- (045)

MM: 70 Time-3Hrs

S. NO	UNIT	SECTION A		SECTION B	SECTION C	SECTION D	SECTION E	Total Marks
		MCQ	Assertion Based Questions	Very Short Answer Questions	Short Answer Questions	Case Based Questions	Long Answer Questions	
		12*1=12 (Q.no 1-12)	4*1=4 (Q.no. 13-16)	5*2=10 (Q.no 17-21)	7*3=21 (Q.no 22-28)	2*4=8 (Q.no-29-30)	3*5=15 (Q.no 31-33)	70
1	RECOMBINANT DNA TECHNOLOGY	4			2		1	15
2	PROTEIN STRUCTURE & ENGINEERING	4	2	1	1	1		15
3	GENOMICS & BIOINFORMATICS	1	1	1	2			10
4	MICROBIAL CELL CULTURE & ITS APPLICATIONS		1			1	1	10
5	PLANT TISSUE CULTURE & ITS APPLICATIONS	3		1	1			8
6	ANIMAL CELL CULTURE & ITS APPLICATIONS			2	1		1	12
TOTAL MARKS		12	4	10	21	8	15	70