



The **'What You will Learn'** section at the beginning of every chapter gives an overview of the topics to be learnt

What we will Learn



- Advantages and limitations of tissue culture
- Equipments required in a tissue-culture laboratory and their utility
- Methodology of handling and starting tissue culture
- Media and serum, their physical and chemical nature
- Quantitation procedure, growth cycle and cell-cycle estimation
- Stem cells and their application in medical and agriculture research
- Somatic-cell fusion, selection of hybrids, and mapping of chromosome using somatic cell fusion protocol

There are three types of restriction enzymes.

1. Type I

These type of restriction enzymes are complex with different sub-units. The enzymes recognise a 15 bp DNA sequence but do not produce a cut within this sequence. The cleavage occurs far away from the recognition site, which may be approximately 1000 bp downstream. The enzymes require ATP, Mg²⁺ and S-Adenosyl methionine as cofactors. These enzymes are not much in use in gene manipulation.

Box 11.3

Case study 2

Discovery of restriction and modification system

Bacteria have the characteristic to destroy incoming DNA, if it is recognised as foreign. When bacteria in a colony are infected with bacteriophage, lysis occurs, thereby causing plaque formation. If the incoming DNA is a bacteriophage DNA the effect is to reduce the number of plaques formed in a plating test, i.e. the effect is to reduce the Efficiency Of Plating (EOP). The phenomenon of restriction and modification were illustrated by the behaviour of phage λ on two *E. coli* host strains.

The λ phage grown upon *E. coli* strain C when titred upon *E. coli* strain K, the efficiency of plating (EOP) is reduced by several orders of magnitude than that of *E. coli* C. It means the phage is *restricted* by *E. coli* strain K. The phages that do result from the infection of *E. coli* strain K are titred upon *E. coli* strain K, and there is no reduction of efficiency of plating, i.e. the phage DNA is no more restricted. But if plated on *E. coli* strain C, there is reduction of plating efficiency. This unalterable change conferred upon the phage by the second host strain (in this case *E. coli* strain K), that allows it not to be further restricted by the strain is called *modification*.

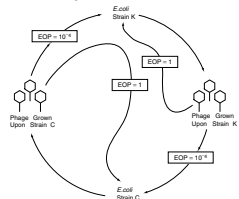


Fig. 11.4 Host controlled restriction and modification of λ phage DNA in *E. coli* strains K and C

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Industry Orientation and Application Orientation

Industrial linkages through CASE STUDIES
Biotechnology laboratory techniques: PCR, Electrophoresis, Chromatography, RDT and Genetic Engineering



Basic Experiments

What we will Learn



- Culture of microbes
- Preparation and maintenance of pure culture
- Characterisation of microbes
- Isolation, purities, culture and characterisation of amylase producing microbes
- Extraction and characterisation of DNA
- Extraction and characterisation of protein
- Tissue culture

28.1

LIST OF EXPERIMENTS

1. To culture microbes in our environment
2. To isolate microbes by streak-plate method
3. To prepare and maintain pure cultures
4. To culture microbes supposed to be industrially important in a mixed culture from different natural sources
5. To isolate strains differing morphologically in colony characters from mixed culture by streak plate
6. To prepare and maintain pure cultures of isolated strains
7. Screening of better strains among isolated strains for amylase activity
8. To culture isolated pure-line strains in broth
9. To characterise and maintain the pure culture of selected strain for amylase activity
10. To study the growth of bacteria in broth by haemocytometer count
11. To find the generation time of pure culture strains isolated from rice water
12. To study the growth of selected strain for amylase activity by spectrophotometric method
13. To study the growth of selected strain for amylase activity in media supplemented with different carbon sources
14. Separation of commercially available DNA and extracted DNA by agarose gel electrophoresis
15. Separations of extracted plant and animal protein by SDS-PAGE
16. To isolate and characterise bacterial genomic DNA
17. To find the effective duration of sterilization to have aseptic culture of carrot cambial tissue

Summary



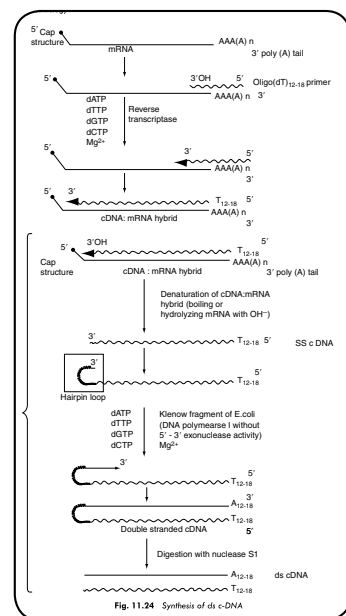
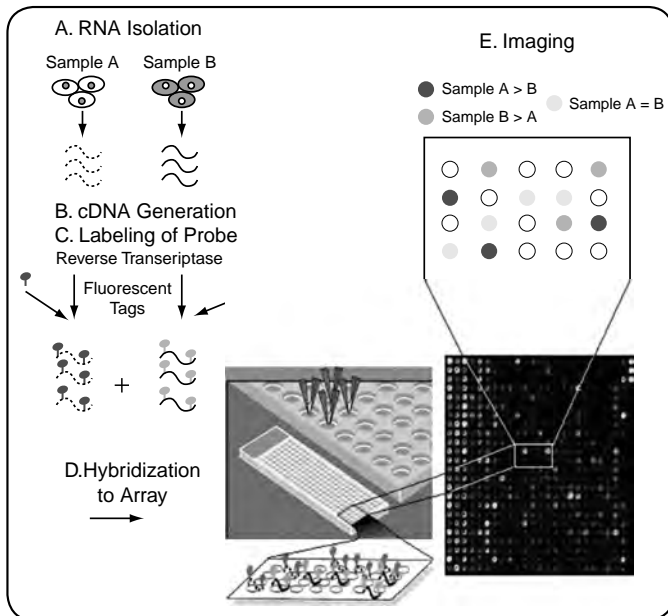
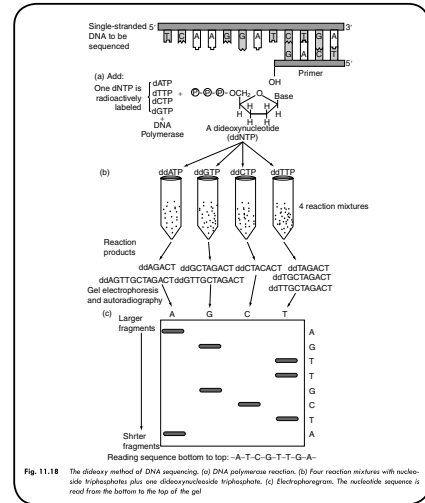
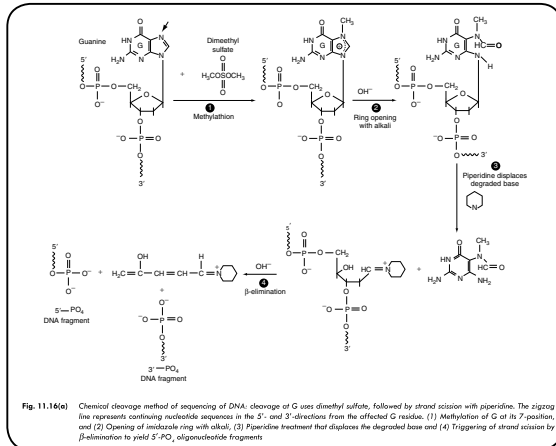
- Enzymes are protein catalysts that regulate the rate of physiological and biochemical processes of living cells.
- They catalyze reactions involving group transfer, isomerization, oxidoreduction or synthesis of covalent bonds.
- Based on the type of reaction, enzymes have been classified into six classes (oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases).
- Enzymes require the assistance of nonprotein coenzymes which are mostly B-vitamin derivatives. Cofactors like metal ions also participate in enzyme-catalyzed reactions.
- Most enzymes are highly specific for their substrates, coenzymes and the types of reactions catalyzed.
- Enzyme activity is measured through techniques like spectrophotometry and electrophoresis. Precise intracellular location of enzymes is done through histochemical and cell-fractionation techniques.
- While almost all enzymes are proteins, catalytic RNAs known as *ribozymes* catalyze specific hydrolysis of phosphodiester bonds in RNAs. *Abzymes* are monoclonal antibodies which in addition to their immunological functions also participate in catalytic reaction.
- The rates of enzyme-catalyzed reactions are regulated by temperature, pH, enzyme concentration and substrate concentration. Activators and inhibitors are also involved to accelerate or inhibit enzyme-catalyzed reactions.
- Enzymes alter reaction rates by *lowering the activation energy*, for the formation of **transition state** and by providing amino acid residues whose functional groups perform specific roles in catalysis at a site, known as *active site* or *catalytic site*.
- Regulation of enzyme activity contributes in a major way to preserve homeostasis. As a result, a relatively constant intracellular and intraorganismal environment is maintained in spite of wide fluctuations in external environment (e.g. temperature).
- The extensive use of enzymes in various fields is the result of enzyme technology. This area embraces production, isolation, use in purified form and immobilization of enzymes. The culture of microorganisms is done in suitable media and the required enzymes are isolated using various methods.
- Immobilization involves attachment of enzymes on insoluble polymers, membranes and particles which act as support or carrier for enzyme activity.
- Immobilization permits reuse of the enzyme after the completion of reaction and formation of products.
- Enzymes are used for therapeutic purposes such as diagnosis of diseases and enzyme therapy. They are extensively used in different industries (e.g. dairy, textile, leather, brewing, detergent, etc.).
- Enzymes are useful in the construction of *biocensors* and *biochips*. They also play a role in pollution control and genetic-engineering techniques.



Each chapter has an extensive **Summary** for quick recapitulation of the concepts discussed.



- More than 600 illustrations and diagrams are present to enhance the concepts.
- Select **Colour** figures add greater clarity to the subject.





Graded chapter-end questions patterned as per university examinations

- **Long-answer questions** to test the student's subjective grasp on the topics
- **Short-answer questions** to test the student's hold on terms and definitions.
- **Objective-type questions** for quick revision and recapitulation of concepts

(A) Long-answer Questions

1. In what way are cells *in vitro* different from cells *in vivo*? What are the major hurdles in growing cells *in vitro*?
2. Design a tissue-culture laboratory. List some major equipment and their uses in a tissue-culture laboratory.
3. While preparing the medium for tissue culture, what physical and chemical parameters should be taken into account? Why are such parameters important?
4. For enzymatic disaggregation which enzyme is most preferred? How can you minimize tissue damage while carrying out enzymatic disaggregation?
5. Give an account of the biology and characters of the cultured cell.
6. Why is it necessary to preserve cells? Describe the method of cell preservation.
7. Discuss the therapeutic use and classification of stem cells.
8. Give an account of aseptic techniques and sterilization procedures for tissue culture.
9. Explain the role of carbon dioxide in maintaining cells *in vitro*.
10. What are major constituents of serum? Why is serum essential in setting some culture? What are its limitations?

(B) Short-answer Questions

1. What are the optimum pH of culture medium for proper cell growth? Which indicator is commonly used to measure the pH?
2. In a tissue-culture laboratory, what are the different types of microscopes essential and why?
3. Why should you insert a cotton plug on the top of a glass pipette before you sterilize it?
4. What are the disadvantages of inclusion of serum in a medium?
5. How does colchicine help in synchronizing cells?
6. Name at least three agents for cell fusion.
7. Name commonly used cryopreservatives.
8. Explain the terms 'cell line' and 'passage'.
9. Name four commonly used commercially available culture media.
10. What are the different phases of cells when they are in a culture medium?

(C) Fill in the Blanks

- i. RNA polymerase holoenzyme consists _____ number of α sub-units.
- ii. The subunit composition of RNA polymerase holoenzyme of prokaryote is written as _____.
- iii. The closed promoter complex is formed due to the formation of complex between RNA polymerase and _____ sequence of DNA.

(D) Multiple choices. Choose the correct answer.

- i. The transfer RNA – tRNA^{met} is able to read the sequence
 - a. AUG, GUG, UUG
 - b. AAG, GUG, UUG
 - c. AAG, GGA, UUG
 - d. AUG, GUG, CCG
- ii. Number of RNA polymerases marked in eukaryotic cells are a-2, b-3, c-4, d-5

Glossary

Acid: A substance that can donate a proton (H ⁺).	Alcohol fermentation: The anaerobic conversion of glucose to ethanol via glycolysis
Activation energy (AC₂): The amount of energy (in joules) required to convert all the molecules in 1 mole of a reacting substance from the ground state to the transition state.	Allele: Alternative form of a gene
Active immunity: Immunity created by a person's own immune system by producing antibodies against infectious agents, e.g. exposure to infectious agents or immunization	Allotric site: The specific site on the surface of an allosteric enzyme molecule to which the modulator or effector molecule is bound
Active site: The region of an enzyme surface that binds the substrate molecule and catalytically transforms it, also known as catalytic site	Amphibolic pathway: A metabolic pathway used in both catabolism and anabolism
Adaptive immunity: An acquired immunity having characteristics of specificity, diversity, memory and discrimination between self and non-self	Amphoteric: Capable of donating and accepting protons
Adapter: A synthetic, double-stranded oligonucleotide, blunt-ended at one end and having a nucleotide extension at the other that can base pair with a cohesive end created by cleavage of DNA molecule with restriction endonuclease II	Antibiotic: A biologically or synthetically produced chemical substance that inhibits growth microorganisms, e.g. penicillin or streptomycin; most antibiotics are not lethal to viruses
ARLP (Amplified Fragment Length Polymorphism): A combination of RFLP and RAPD, very sensitive in detecting polymorphism in a genome	Antibody: A protein produced by the immune system of an animal in response to a foreign substance called antigen; also known as immunoglobulin (I), each antibody molecule is composed of 4 sub-units of two heavy chains and two light chains
Agar: A glutinous polysaccharide that forms a gel with water and solidifies when allowed to stand at room temperature; used extensively as a culture medium for the growth of bacteria and fungi derived from some red algae of the genus Gelidium, Gracilaria, Gigartina, Alginella	Antigen: A protein (immunoglobulin) synthesized by a B lymphocyte and recognizes a specific site on an antigen
	Antigen: A compound that induces the production of antibodies; it elicits an immune response when introduced into an animal, and is specifically recognized by an antibody
	Assay: A test to detect the presence of some substances in a small amount in solution

A Glossary is provided at the end of the book to help students look up the definitions of important terms and concepts.

