

**Introduction of the Course**

This course work is designed to highlight importance of Gene Cloning in the field of research and Biotechnology including learning about the concept of cloning agents, their behavior, structure and manipulations. It will help the students to learn advance techniques regarding the synthesis of recombinant DNA and their manipulations for the production of beneficial products such as hormones and metabolites. It will also be helpful for students to learn applications of these techniques in various fields such as agriculture and medicine.

**Course Objectives**

1. To introduce advance genetic engineering techniques to the students
2. To enable the students to understand various DNA manipulations at the molecular level

**Contents:****Unit –I: The Principles of Cloning DNA:**

- 1.1. General Principles of Cloning
- 1.2. Strategies for gene cloning

**Unit-II Vehicles: Plasmid and Bacteriophages:**

- 2.1 Plasmids
  - i. Basic Features of Plasmids
  - ii. Size and Copy Number
  - iii. Conjugation and Compatibility
  - iv. Plasmid Classification
- 2.2. Bacteriophages:
  - i. Basic Features of Bacteriophages
  - ii. Lysogenic Phages
  - iii. Viruses as Cloning Vehicles

**Unit-III: Purification of DNA:**

- 3.1. Preparation of total Cell DNA
- 3.2. Preparation of Plasmid DNA
- 3.3. Preparation of Bacteriophage DNA

**Unit-IV: Manipulation of Purified DNA:**

- 4.1. The range of DNA Manipulative Enzymes
- 4.2. Enzymes for Cutting DNA- Restriction Endonucleases
- 4.3. Ligation- Joining DNA Molecule together

**Unit-V: Introduction of DNA into Living Cells:**

- 5.1. Transformation
- 5.2. Selection for Recombinants
- 5.3. Introduction of phage DNA into Bacterial Cells
- 5.4. Selection for Recombinant Phage

**Unit-VI: The Applications of Cloning in Gene Analysis:**

- 6.1. Cloning of Specific Gene
- 6.2. Studying Gene Location and Structure
- 6.3. Studying of Gene Expression

**Unit-VII: Gene Cloning in Research and Biotechnology:**

- 7.1. Production of Protein from Cloned Gene
- 7.2. Gene Cloning in Medicine
- 7.3. Gene Cloning in Agriculture

**Practicals:**

1. Problems related to gene cloning
2. Conjugation
3. Total cell lysate preparation
4. Plasmid DNA Isolation
5. Plasmid DNA detection on Gel Electrophoresis
6. Transformation of Plasmid DNA to *E. coli*.

**Teaching-learning Strategies**

1. Lectures
2. Group Discussion
3. Laboratory work
4. Seminar/ Workshop

**Assignments Types:**

1. Lecture Based Examination (Objective and Subjective)
2. Assignments
3. Class discussion
4. Quiz
5. Tests

**Assessment and Examinations:**

As per University Rules

**Recommended Readings**

1. Brown, T. A. (2020). *Gene cloning and DNA analysis: an introduction*. John Wiley & Sons. 8<sup>th</sup> ed.
2. Fowler, S., Roush, R., Wise, J., & Stronck, D. (2013). *Concepts of biology*. OpenStax College, Rice University.
3. Gardner, E.J. (2004). *Principles of Genetics*. John Willey and Sons, New York.
4. Glover, D.M. (2001). *Gene Cloning. The Mechanics of DNA Manipulation*. Chapman and Hall.
5. Glover, D. M. (2013). *Gene cloning: the mechanics of DNA manipulation*. Springer.
6. Pierca, B.A. (2005). *Genetics; A Conceptual Approach*. W. H. Freeman and Company, New York.
7. Primrose, S.B., Twyman, R.M. and Old, R.W. (2004). *Principles of Gene Manipulation, an Introduction to Genetic Engineering*. (6<sup>th</sup> Ed.), Blackwell Scientific Publications.
8. Primrose, S.B., and Twyman, R. M. (2006). *Principles of Gene Manipulation and Genomics*. Blackwell Scientific Publications.
9. Roy D. (2010). *Biotechnology*. Alpha Science, Technology & Engineering
10. Snustad, D.P. and Simmons, M. J., (2005). *Principles of Genetics*, (4<sup>th</sup> Ed.). John Wiley and Son, Inc. New York.
11. Stacey, J. (2010). *The cinematic life of the gene*. Duke University Press.
12. Synder, L. and Champness, W. (2004). *Molecular Genetics of Bacteria*. ASM Press, Washington D.C.
13. Wilson, J. and Hunt, T. (2004). *Molecular Biology of the Cell – The Problems book*, Garland Publishing Inc.

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