## Isolation and characterisation of Plasmid DNA

### CREDIT DISTRIBUTION, ELIGIBILITY AND PRE-REQUISITES OF THE COURSE

| Course title & Code | Credits | Credit distribution of the course | | | Eligibility criteria | Pre-requisite of the course (if any) |
|---|---|---|---|---|---|---|
| | | Lecture | Tutorial | Practical/ Practice | | |
| **Isolation and characterisation of Plasmid DNA** | **2** | | **NIL** | **2** | **Class XII** | **NIL** |

**Learning Objectives:**

Students of this course should be able to learn:

- Fundamentals of nucleic acid molecules.
- Handling and growing of non-pathogenic bacterial strains of E. coli for recombinant DNA work.
- Basics of Plasmids and its isolation from the culture using different methods.
- Basics of electrophoresis techniques employed for the separation of Nucleic acid molecules.

**Learning Outcomes:**

At the end of this course, students should be able to learn and perform in Hands-on mode:

- Fundamentals of operation of different types of centrifuges, Electrophoresis, Spectrophotometer and about Good Laboratory Practices and working environment of Genomic Laboratory.
- Use of micropipettes, preparation of solutions, media and sterilisation.
- Basics of different types of nucleic acids
- Handle bacterial strains of E. coli for the isolation of single colony and growth in liquid media.
- Isolate Plasmids using different methods and characterise by agarose gel electrophoresis.

**Unit 1: Basic microbiological techniques for culturing and growth of bacteria (16 hours)**

Information on general and molecular biology laboratory practices including Biosafety, Information about important strains E. coli used in recombinant DNA work; chemical composition of media used for growing E. coli both on solid surface and in liquid culture.

**Practical:**

1.1 Pipetting using macro and micro pipettes, macro and micro weighing, measurement of pH, preparation of buffers and other solutions.

1.2 Preparation of solid and liquid media for growing E. coli, sterilization using autoclave and use of Biosafety cabinet.

1.3 Pouring of Petri plates with solid agar media and streaking of E. coli to isolate single colonies.

1.4 Inoculation of E. coli from streaked plate to obtain grown culture.

**Unit 2: Isolation of Plasmids and their characterisation (32 hours)**

Definition and Features of a plasmid, Comparative description of different plasmids in respect of copy number, compatibility and antibiotics resistance markers, various plasmid isolation methods, Gel electrophoresis for nucleic acids.

**Practical:**

2.1 Isolation of plasmid DNA using the available culture by alkaline lysis method

2.2 Preparation of agarose gel, electrophoresis and visualization of plasmid DNA on the gel using transilluminator, characterisation of different forms of plasmid DNA.

**Unit 3: Isolation and characterization of plasmid DNA by use of column (16 hours)**

Technology for isolation of nucleic acids using column, principle of binding and elution of DNA from column. Chromatographic techniques for isolation of nucleic acids.

**Practical:**

3.1 Isolation of plasmid DNA using the self-grown culture by spin column method.

3.2 Preparation of agarose gel, electrophoresis and visualization of plasmid DNA on the gel, characterisation of different forms of plasmid DNA.

3.3 Documentation of the gel using gel documentation system.

**Essential/ Recommended Readings:**

1. Sambrook J, Fritsch EF & Maniatis T. Molecular Cloning. A laboratory Manual. 3rd Edition. Cold Spring Harbor Laboratory Press. New York.

**Suggestive Reading:**

1. Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K. Current Protocols in Molecular Biology. (eds.) John Wiley & Sons, Inc. New York.

**Note:** Examination scheme and mode shall be as prescribed by the Examination Branch, University of Delhi, from time to time.