7. Recombinant DNA technology workshop

Course details

Lecture 1: Introduction to Recombinant DNA technology

- Introduction to gene cloning
- Importance and application of gene cloning
- Case study-recombinant Insulin production

Lecture 2: Types of vectors

- Importance of vectors
- Essential components of vectors
- Types of vectors
    - Cloning vector-characteristics
    - Expression vector-characteristics
    - Shuttle vectors
- Selection of appropriate vector

Lecture 3: DNA manipulating enzymes

- DNA manipulation- overview
- Restriction endonucleases
    - Discovery
    - Characteristics
    - Mechanism
    - Types
    - Applications
    - RM system and its importance
- DNA Ligases
    - Discovery
    - Characteristics
    - Mechanism
    - Types
    - Applications

Lecture 4: Preparation of vector DNA

- Plasmid DNA isolation (manual and kit method)
- Quantitation and visualization
- Restriction digestion of vector DNA
- Purification of digested vector DNA (kit method)

Lecture 5: Preparation of Insert DNA

- Genomic DNA isolation (manual and kit method)
- Quantitation and visualization
- Primer designing for cloning
- PCR amplification of gene of interest (GOI)
- Restriction digestion of GOI
- Purification of GOI (kit methods)

Lecture 6: Ligation

- Ligation reaction-mechanism

- Ligation reaction components and reaction set up
- Vector to insert ratio calculation
- Controls and troubleshooting

Lecture 7: Transformation

- Transformation- introduction and types
- Chemical transformation
    - Mechanism
    - Reaction set up and workflow
    - Controls and troubleshooting
- Electroporation
    - Mechanism
    - Reaction set up and workflow
    - Controls and troubleshooting
- Detailed protocol
    - Competent cell preparation
    - Transformation- Chemical/electroporation
    - Recovery
    - Selection

Lecture 8: Selection of right clones

- Selection-why?
- Various selection methods
    - Blue white selection- concept and methodology
    - Restriction digestion-- concept and methodology
    - Colony PCR-- concept and methodology
    - Sequencing-- concept and methodology

Lecture 9: Overexpression and Production of recombinant proteins

- Different expression systems and their characteristics
    - Bacterial expression system
    - Yeast expression system
    - Mammalian expression system
    - Insect expression system
- Mechanism of overexpression
- pET system -overview, concept and mechanism
- Experimental workflow for production of recombinant proteins
- Controls and troubleshooting

Course Details

Lecture 1: Introduction to antigen, antibody and epitope

- Concept of antigen and epitope
- Antibody structure
- Monoclonal verses polyclonal antibody
- Primary verses secondary antibody

Lecture 2: Introduction to Immunoassays

- Antigen antibody reaction

- Immunoassay- concept and overview
- Immunoassay-types and mechanism

Lecture 3: Immunoelectrophoresis

- Immune complex formation
- Precipitation reaction
- Precipitation curve
- Application of precipitation reaction
- Immunodiffusion
    - Radial immunodiffusion
    - Double immunodiffusion
- Immunoelectrophoresis
        - Rocket immunoelectrophoresis
        - Counter immunoelectrophoresis

Lecture 4: Immunoblotting traditional method

- Immunoblotting- Concept, history and overview
- Detailed methodology with demonstration by video
    - Sample preparation
    - SDS-PAGE
    - Transfer and blocking
    - Blot development
    - Detection analysis
- Application and troubleshooting

Lecture 5: Immunoblotting with SNAP i.d

- Limitations of traditional immunoblotting method
- Advantage of SNAP i.d.
- Mechanism
- Components of SNAP i.d.
- Experimental setup with demonstration by video
- Control and troubleshooting

Lecture 6: Radioimmunoassay (RIA)

- Principle of RIA
- Discovery and applications
- Ria-Types
- Experimental setup and workflow
- Limitations and advantages

Lecture 7: Enzyme-linked immunosorbent assay (ELISA)

- Antibody and Antigen
- Immunoassay
- Enzyme linked immunosorbent assay (ELISA)
- Types of ELISA
- Applications of ELISA
- Experimental setup and workflow

- Advanced Immunoassay

Lecture 8: Immunohistochemistry (IHC)

- Principle of IHC
- Immunoblotting verses IHC
- Experimental workflow with demonstration by video
    - Sample preparation
    - Antigen retrieval
    - Blocking
    - Stanning
    - Visualization
- Applications