Seminars Practical works

Modern methods of biotechnology

…. Autumn semester 1 course.

7M05109 Biotechnology

7M05114

2025 teaching year

Objectives of the discipline:

To acquaint Ms students with the peculiarities of the essence and applications of the main methods used in modern biotechnology in studying processes at the various level of organization of living organisms and to show their relationship with the purpose of research, to form the ability and skills to apply the basic methods of research in cellular and molecular biotechnology, microbiology, used in the analysis of biological objects and products obtained in the implementation principles and use the latest trends and technologies to obtain targeted genetically modified or engineered products for various fields of application.

Learning outcomes and competences:

1. To describe the principles and applications of main methods of cellular approaches used in biotechnology including cellular membrane structures, study of nucleic acids and proteins, genetic engineering, to analyze information when deciding practical tasks.
2. 2. To classify and determine the key biophysical methods for studying cell membrane structures.
3. 3.To determine the key biochemical methods for studying cell membrane structures
4. . To demonstrate the knowledges and principles of main proteome methods ; describe the schemes used to characterize their basic area of application.
5. 5. To interpret the molecular methods used for research and practical purposes of biotechnology.
6. 6. To solve the main methods of genetic engineering.

1.1.To determine the key biochemical methods for studying cell membrane structures.
1.2. To analyze the methods Isolation of cell membrane fractions
2.1. Explains how nuclear magnetic resonance spectroscopy (NMR spectroscopy) method. Surface plasmon resonance (SPR).
2.2. Demonstrates knowledges methods to study dynamics and interactions of biological membranes.
2.3. Shows methods for determining the electrical characteristics of membranes. Defines Biochemical methods for studying biological membranes. Identification of cellular membrane fractions by microscopic analysis
3.1. Indicates methods of solubilization and reconstruction of membranes
3.2. Shows methods of isolation of cell membrane fractions.
3.5. makes final conclusions from the data obtained;
4.1. Classifies Ion-exchange chromatography,
Affinity chromatography,
Protein electrophoresis
4.2 Illustrates determination of protein composition, marker enzyme activity and cell surface protein biotinylation for identification of cellular membrane fractions

5.1. Shows general principles of nucleic acid isolation Isolation of total DNA from plant tissues.
Isolation of total DNA from plant tissues
Total DNA isolation method using 5 M potassium acetate. Total DNA isolation method using

CTAB detergent.; 5.2 iscovers methods of the features of the essence for extraction, modification, purification and analysis of nucleic acids. Hybridization and nucleic acids various blotting techniques Method of Southern blotting The Northern blotting method. DNA microarray. Reverse northern blotting RNA-seq (Next-Gen sequencing) Single-molecule real-time RNA sequencing. Single-cell RNA sequencing (scRNA-Seq)..
6.1. Illustrates RNA interference (RNAi) method and synthetic biology and de novo gene synthesis.
6.2. Explains how Recombinant DNA (rDNA) technology used for genetic improvement of living organisms. Applications of polymerase chain reaction (PCR). Gene editing (CRISPR-Cas9). Gene transfer techniques. Direct transformation (non-viral methods) method and no direct transduction (viral-mediated gene transfer).

Contents

Practical class 1. Theme. Modern biotechnology for food and agriculture. Application and trends of biotechnology in sustainable agriculture. Evolution' of agricultural biotechnology. Development of new products by using different modern approaches

Practical class 2. Theme Determination of protein composition, marker enzyme activity and cell surface protein biotinylation for identification of cellular membrane fractions. Crude isolation of plant plasma Membrane by differential centrifugation. Differential centrifugation and their use. Methods of identification of subcellular fractions.

Practical class 3 Theme. Methods for determining the electrical characteristics of membranes. Radioisotope uptake assays. Stopped-flow system. Methods of studying ions channels. Solubilization and reconstruction of membranes. Types of microscopies.

Practical class 4. Theme. Methods of solubilization and reconstruction of membrane structures

Practical class 5. Theme Western blotting method (protein immunoblot). Describe enzyme activity analysis by electrophoresis. Different types of electrophoresis and gel visualization. Consider the protocols for these methods. Describe methods in study of proteins separation according to distinct physical properties. Show preparative gel electrophoresis in protein study. Calculation of molecular mass from SDS gels Describe enzyme activity analysis by electrophoresis Show preparative gel electrophoresis in protein study. Practical exercises include protocol of preparation of gel, reaction mix for determination of enzyme activity.

Practical class 6. Protein-next generation sequencing. Protein N-terminal amino acid analysis. Protein C-terminal amino acid analysis. Edman degradation sequencing peptides and proteins

Practical class 7. Isoelectric focusing, two-dimensional electrophoresis for protein separation (2DE). Isotachophoresis. Methods for quantitative determination of proteins

MODULE 2. Molecular methods used in biotechnology

Practical class 8. Theme. Isolation of total DNA from plant tissues. Total DNA isolation method using 5 M potassium acetate. Total DNA isolation method using CTAB detergent. Method for isolating total DNA from plants with increased phenol content. Method for isolating chloroplast and mitochondrial DNA. Main principles of RNA isolation from different biological organisms. Consider plant protein expression, purification and characterization including:

1. . RNA isolation.

2. cDNA synthesis.
3. qPCR uses SYBR Green and TaqMan probes**.**

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Practical class 9. Theme. Single-molecule real-time RNA sequencing. Single-cell RNA sequencing (scRNA-Seq). DNA sequencing methods. Sanger dideoxy (primer extension/chain-termination) DNA sequencing Microfluidic Sanger sequencing. Maxam–Gilbert sequencing-DNA next-generation sequencing. Next-generation sequencing platforms

Practical class 10. Types of real time PCR. Absolute and relative quantification of cDNA. Method of serial analysis of gene expression (SAGE) and DNA microarray.

SAGE is a technique used to create a library of short sequence tags which can each be used to detect a transcript. The expression level of the transcript can be determined by assessing how many times each tag is detected. This technology enables comprehensive expression analysis across the genome.

DNA microarray

Also known of as biochip or DNA chip, a DNA microarray is a solid surface to which a collection of microscopic DNA spots are attached. The microarrays are used to determine expression levels across a large number of genes or to perform genotyping across different regions of a genome.

Practical class 11. Modern types of DNA markers in agriculture. Advantages and main principles for for choice of DNA markers

KASP markers (Kompetitive allele specific PCR). RNA-based molecular markers. Types.

The types of protein based molecular markers in biotechnology. Genome-wide association study (GWAS). Types. Sample of genome-wide association study (GWAS) protocol.

Practical class 12. Theme. Basic principles and methods genetic engineering. Choice of host organisms and vectors for genetic engineering methods.

Genetically modified organisms such fungi, viruses, plants, crops, animals, gene therapy and for bioremediation

P Practical class 13. Theme. Plant metabolic engineering. Approaches to plant metabolic engineering. Biomolecular engineering. Biomolecular engineering for manipulation of carbohydrates, proteins, nucleic acids and lipids.

Practical class. 14. Theme. Methods of RNAi delivery in cells**.** The methods of RNAi delivery in cells including the various approaches: Applications of gene function studies – applied in functional genomics to study gene roles, antiviral therapy – RNAi targets virapplies like *HIV,* Hepatitis B, and SARS-CoV-2, cancer research and agricultural biotechnology.

Practical class 15. Examples for cell and tissue engineering. Bioartificial organs. Stem cells.

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