MOLECULAR BIOTECHNOLOGIES (A000208)

1. language

Italian.

2. course contents

Coordinator: Prof. ARCOVITO ALESSANDRO

Academic Year: 2022/2023

Year Course: 1

Semester: 1

UFC: 11

Modules and lecturers:
- BIOCHIMICA ANALITICA (A000226) - 3 cfu - ssd BIO/10
Prof. Alessandro Arcovito, Giuseppina Nocca, Federica Iavarone
- BIOTECNOLOGIE MICROBICHE (A000229) - 3 cfu - ssd MED/07
Prof. Giovanni Delogu, Francesca Bugli
- PROTEINE RICOMBINANTI (A000227) - 3 cfu - ssd BIO/11
Prof. Concetta Santonocito, Andrea Urbani
- TECNICHE DI TRASFERIMENTO GENICO E TERAPIA GENICA (A000228) - 2 cfu - ssd MED/03
Prof. Eugenio Sangiorgi

3. bibliography

BIOCHIMICA ANALITICA

- Metodologie biochimiche e biomolecolari. Strumenti e tecniche per il laboratorio del nuovo millennio. Mauro Maccarrone Zanichelli (2019)

PROTEINE RICOMBINANTI
- Biotecnologie Molecolari principi e tecniche (Terry A. Brown) – Zanichelli 2017

TECNICHE DI TRASFERIMENTO GENICO E TERAPIA GENICA
- Mauro Giacca, Terapia genica Springer 2011
BIOTECNOLOGIE MICROBICHE
- Biotecnologie Microbiche – S. Donadio, G. Marino - Casa Editrice Ambrosiana

Additional didactic material will also be provided, in the form of scientific articles and appropriate telematic sources, to complete, deepen and update the contents covered in class.

4. learning objectives

The integrated course aims to deepen the specialist knowledge of topics related to modern molecular biotechnologies in the field of high-throughput analytical biochemistry techniques, genetic engineering methods and molecular biology for the production of recombinant proteins and

gene therapy applications.

At the end of the integrated course the student must demonstrate that he has acquired the following objectives:

Knowledge and understanding - demonstrate knowledge and ability to understand the problems related to modern Molecular Biotechnologies necessary for the characterization of omic profiles associated with human pathologies and for the development of modern genetic engineering techniques, through the understanding of gene recombination mechanisms in microorganisms and their use in biotechnological systems

Applied knowledge and understanding - demonstrate knowledge of adequately interpreting and understanding the application implications of Molecular Biotechnology, highlighting its translational potential in the diagnostic and therapeutic field, through the identification of the genetic determinants of biological processes, with particular reference to the mechanisms of microbial pathogenicity, for the development of recombinant vaccines and the development of innovative biological therapies.

Autonomy of judgment - knowing how to integrate the knowledge and skills learned to identify the most appropriate Molecular Biotechnologies for determining disease diagnostic / prognostic profiles and for the development of innovative therapeutic protocols.

Communication skills - knowing how to communicate clearly and without ambiguity, correctly using the technical language, one's conclusions as well as the knowledge and rationale underlying them to specialist and non-specialist interlocutors.

Ability to learn - to be able to update oneself and to broaden one's knowledge by independently drawing on texts, scientific articles and online platforms and databases (NCBI, Ensemble, UniProt, PDB etc). Must gradually acquire the ability to attend specialized seminars, conferences, masters, etc.

5. PREREQUISITES

Students must have acquired the knowledge relating to the basic disciplines envisaged in the three-year degree courses preparatory to this degree class, with particular reference to: Biochemistry, Molecular Biology, Microbiology and Genetics.

6. teaching methods

The teaching methodology is based on lectures delivered by providing both the basic elements of the various disciplines and the applicative perspectives. The lessons are based on interactive methods, integrating activities based on active learning to standard teaching, such as: "problem-based learning", "self-learning", and "case studies".

The teaching methods used in this course are designed to allow the student to pursue the educational objectives, by virtue of the following characteristics:

Knowledge and understanding - frontal teaching will deal systematically all the topics listed in the program detailed below, focusing on the most relevant and essential aspects, in order to provide students with the complete picture of the integrated topics and the correct study method to strengthen theoretical knowledge.

Applied knowledge and understanding - the use of practical examples, classroom exercises and case studies allows students to learn the applicative potential of the topics covered. In particular,

for the Applied Biochemistry module, students will be offered situations in which they will have to decide which analytical techniques to use to solve some scientific questions.

Independent judgment - the active learning methods implemented in this course are designed to allow the student the ability to formulate concepts and ideas independently.

Communication skills - active learning methods and constant interaction with the teacher during lectures will be conducted in a way that allows the student to gradually acquire communication skills aimed at exposing chemistry and biology topics with the correct scientific terminology.

Ability to learn - the use of supplementary teaching material, including in the form of articles from international scientific literature, will allow the student to undertake subsequent studies with a high degree of autonomy.

ATTENTION: In the event that the problems relating to the Covid-19 health emergency do not make it possible to carry out the lessons in person, these will be carried out in synchrony respecting the published timetable and using the Blackboard platform.

7. other informations

Experimental degree theses to be carried out in the research laboratories of the Catholic University of the Sacred Heart and the A. Gemelli University Hospital Foundation - IRCCS are available at the Departments and the relative Sections to which the lecturers of the course belong. .

Teachers receive by appointment agreed via e-mail.

Prof. Alessandro Arcovito alessandro.arcovito@unicatt.it

Prof. Giuseppina Nocca giuseppina.nocca@unicatt.it

Prof. Federica Iavarone federica.iavarone@unicatt.it

Prof. Andrea Urbani andrea.urbani@unicatt.it

Prof. Concetta Santonocito concetta.santonocito@unicatt.it

Prof. Giovanni Delogu giovanni.delogu@unicatt.it

Prof. Francesca Bugli francesca.bugli@unicatt.it

Prof. Eugenio Sangiorgi eugenio.sangiorgi@unicatt.it

8. methods for verifying learning and for evaluation

The exam consists of an oral exam, which will focus on the contents of the course modules and which will be awarded a score resulting from the weighted average of the result obtained in each module. The maximum final score (30/30 cum laude) will be awarded only in the presence of unanimous judgment by the Commission.

The objective of the exam is to evaluate the student's acquisition of the following skills and knowledge:

Knowledge and understanding - of the appropriate level of knowledge of the topics covered by the

program and an understanding of the role of the various molecular techniques studied;

Applied knowledge and understanding - of the ability to connect theoretical concepts of microbiology, biochemistry and molecular biology to the diagnostic and therapeutic field

Autonomy of judgment - the ability to make transversal connections on the topics covered;

Communication skills - adequate command and ownership of language and correct technical / scientific terminology;

Ability to learn - the ability to investigate issues of molecular biotechnological interest independently and to use critical reasoning.

ATTENTION: If the problems relating to the Covid-19 health emergency do not make it possible to carry out a test in person, the exam will be carried out orally using the Blackboard platform. The student will be questioned by at least one teacher per module delivered. The final grade will be given by the commission in collegial form and will go from a minimum of 18/30 to a maximum of 30/30. Honors will be given by a unanimous vote of the examination commission.

9. program

<Analitical Biochemistry>

Chromatographic techniques: general principles, resolution and theoretical plates. Column chromatography. Adsorption and partition chromatography. Notes on TLC: two-dimensional chromatography. Protein ion exchange chromatography. Exclusion chromatography (gel filtration). Affinity chromatography. Immunoaffinity. HPLC. Mass spectrometry techniques: Introduction to mass spectrometry and general principles; Ion sources: hard and soft ionization techniques. Mass analyzers: quadrupole, ion trap, time of flight, magnetic and electromagnetic sector. Fourier transform mass spectrometry: Orbitrap and cyclotron ion resonance analyzer. Hybrid tools. Detectors and data acquisition Coupling of mass spectrometry with chromatographic, gas and liquid techniques.

Introduction to absorption and fluorescence spectroscopy. Electrophoretic techniques: general principles, factors influencing electrophoretic mobility. Free phase electrophoresis. Zonal electrophoresis on cellulose acetate; serum proteins. Gel electrophoresis: PAGE, PAGE-SDS, Agarose. Methods of detection and quantitative evaluations. Blotting - Western blotting in the study of proteins: biochemical and diagnostic applications. Isoelectric Focus (IEF). Capillary electrophoresis Two-dimensional electrophoresis. Spectroscopic techniques for determining bond affinity: surface plasmon resonance and stopped flow.

Immunochemical techniques. Outline of antibody structure and antigen-antibody reaction. Methods of analysis: free phase and gel immunoprecipitation reaction, immunodiffusion; radioimmunological methods: RIA; enzyme immunoassays, ELISA. General aspects of laboratory tests: analytical and diagnostic characteristics (e.g. biological variability, reference values, desirable values and decision levels) typology of the different biological samples and related sampling methods (preparation of the patient for sampling; factors that influence analytical parameters). Analytical and post-analytical variability. Data reliability criteria. Precision and accuracy. Types of errors and their causes. Repeatability and reproducibility. Standard deviation and coefficient of variation (CV), LOQ, LOD. Precision and accuracy; Analytical and diagnostic specificity and sensitivity. Predictive values. Prevalence and incidence. Evaluation of an analytical method. Characteristic operating curve (ROC).

<Proteine ricombinanti>

Production of recombinant proteins: Introduction to the production of recombinant proteins; Cloning techniques and expression vectors; "Cell free" systems for the expression of proteins; Expression in prokaryotic systems (Gram negative and Gram positive); Expression in E. coli; Fermentation of E. coli: optimization of the process with genetic and physiological tools .; Strategies for increasing the expression of soluble proteins: procedures to be adopted to minimize the formation of inclusion bodies; Expression in eukaryotic systems: Pichia, Saccharomyces; Expression in eukaryotic systems: insect and mammalian cells.

Purification: Sample preparation. Extraction and clarification. Methods of cell disruption and production of the initial crude extracts. Protein solubilization methods. Saline swabs. Fractionation and precipitation techniques. Filtration, dialysis, sample concentration. Preservation of the purified protein.

Protein Engineering: rational design de novo protein design (of structures and functions): methods and examples Rational design: theory and experimental methods. Molecular docking examples: theory and examples Protein stability engineering: theory, methods and examples

<Tecniche di trasferimento genico e terapia genica>

- Introduction: Generalities, definitions, brief history of gene therapy.

- Main gene therapy applications of hereditary diseases (hematopoietic stem cell diseases, cystic fibrosis, haemophilia, muscular dystrophy, thalassemia).

- Gene therapy of tumors: specific aspects of gene therapy of tumors. Transduction of normal cells and cancer cells. Vectors for proliferating cells. Transduction efficiency and bystander effect. Gene therapy of the transformed phenotype. Genes overexpressed in cancer cells. Gene therapy of tumor neoangiogenesis. Gene immunotherapy of tumors.

- Gene therapy for neurodegenerative diseases: Parkinson's disease, Alzheimer's disease, ALS, Huntington's chorea

< Biotecnologie Microbiche>

Gene recombination mechanisms in bacteria and genetic variability. The bacterial genome: characteristics and generalities. Plasticity of the bacterial genome. Genomics, comparative and functional genomics.

Molecular epidemiology: principles and applications.

Recombination and Recombineering. Molecular basis of site-specific and homologous recombination. The Lambda Red system and its applications: allelic exchange; gene knock-out; epitope- tagging. Transposon mutagenesis. Signature-tagged mutagenesis. CRISPR.

Gene reporter technology: IVET, RIVET ,. Principles, systems and applications.

Mechanisms of microbial pathogenicity. Regulation of virulence genes. Secretion systems. Identification of virulence factors.

Eukaryotic vectors. DNA vaccination: principles, mechanism of action, methodologies, applications. Replicative vectors. Alphaviruses as vectors; The replicative plasmids.

Live and attenuated vaccines: Viral systems as tools for the development of new vaccines; principles, methodologies, applications.

Live and attenuated vaccines: Salmonella, Shighelle, Vibrio, BCG; principles, methodologies and applications.

Subunit vaccines: historical notes, principles, methodologies and applications. Reverse vaccinology: principles and applications. The examples of Neisseria meningitidis and Streptococcus agalactiae. Transgenic plants: principles and applications. Phages and phage therapy.