Experimental Biotechnology Laboratory (A000214)

1. LANGUAGE

Italian.

2. COURSE CONTENTS

Coordinator: Prof. PALACIOS GARCIA DANIELA

Academic Year: 2022/2023

Year Course: 1

Semester: II

UFC: 12

Modules and lecturers:
- ISOLATION AND CARACHTERIZATION OF STEM CELLS (A000250) - 3 cfu - ssd BIO/13
Prof. Daniela Palacios Garcia, Andrea Papait, Ornella Parolini, Valentina Saccone
- GENOMIC MEDICINE (A000251) - 3 cfu - ssd MED/03
Prof. Serena Lattante
- ADVANCED MICROSCOPY (A000248) - 3 cfu - ssd FIS/07
Prof. Massimiliano Papi
- CLINICAL PROTEOMICS AND METABOLOMICS (A000249) - 3 cfu - ssd BIO/12
Prof. Silvia Persichilli, Andrea Urbani

3. BIBLIOGRAPHY

Isolation and characterization of stem cells: updated scientific articles, journals and protocols provided to students during the course.

Genomic Medicine: scientific articles and protocols provided to the students during the course.

Advanced Microscopy: D. Chandler e R. Roberson: BIOIMAGING. Jones & Bartlett Publishers ISBN: 128406316X

Clinical Proteomics and Metabolomics: "Proteomica" , Alberio Fasano, Roncada, EDISES, ISBN 9788836230495

Where necessary, students will be provided with supplementary teaching materials, in the form of articles, journals and protocols updated during the course, as well as the indication of appropriate and reliable telematic sources, to supplement, deepen and update the content explained in class.

4. LEARNING OBJECTIVES

The integrated course aims to provide the student with practical laboratory skills in the different areas of the course, through the implementation of series of exercises focused on learning how to use different instrumentation and associated experimental protocols.

Upon completion of the integrated course, the student must demonstrate that he or she has

acquired the following objectives.

Knowledge and Understanding - *Demonstrate knowledge and ability to understand: the principles of proteomics and metabolomics essential for experimental analysis and interpretation of results in the field of biochemical and molecular biological disciplines, possible applications of genetic and molecular technologies necessary for the analysis of DNA, RNA and proteins, the main sources and methods for the isolation and in vitro characterization of stem cells. The student must demonstrate knowledge and ability to interpret images/data acquired by advanced microscopy techniques such as atomic force microscopy and advanced confocal microscopy and demonstrate the ability to identify the most appropriate microscopy technique depending on the scientific question.*

Applied knowledge and understanding skills - *demonstrate the ability to adequately interpret and understand the applications of experimental biotechnology by mastering their use and developing the critical capacity for their most appropriate application in both the diagnostic and research fields. Specifically, the student will learn how to read and interpret a scientific protocol and draft the laboratory notebook to clearly and correctly report the experiments performed, the use of: 1)proteomics, interactome and metabolomics techniques, gaining knowledge of the main separation methods (2D gel, HPLC) and will have the opportunity to use high-performance protein and metabolite identification instrumentation such as mass spectrometry; 2) genetics and genomics techniques and bioinformatics programs for analysis of gene variants and their functional implication on mRNAs and proteins; 3) advanced microscopy techniques for ultrastructural analysis of cells and tissues and data analysis; 4) techniques for isolation, culture and phenotypic and functional characterization of somatic stem cells from human tissues.*

Autonomy of judgment - *know how to integrate the knowledge and skills learned in the different modules and acquire critical and design skills in order to independently carry out observations and experiments based on the laboratory activities learned.*

Communication skills - *to be able to describe clearly and unambiguously, using correctly the technical language specific to the analytical methodologies learned, the protocols learned during laboratory activities. The student will also develop the ability to work in groups and clearly communicate their knowledge or research results, both to an audience of specialists and to a wider audience of non-specialists.*

Ability to learn *- independently select appropriate sources for the development of experimental protocols, drawing on advanced texts and telematic resources, in Italian and English.*

5. PREREQUISITES

It is necessary for students to have acquired knowledge related to the basic disciplines provided in the three-year degree courses preparatory to this graduating class, with special reference to theoretical aspects and acquisition of basic laboratory practical experience in the following disciplines: biochemistry, molecular biology, cell biology and genetics6. Metodi didattici/teaching

methods

6. TEACHING METHODS

The teaching methodology is based on theoretical-practical exercises and experimental activity, laboratory exercises, and complements standard teaching with activities marked by active learning, such as: "problem-based learning," "self-learning," and "case study," "virtual laboratory," presentation of a scientific work selected from recent literature.

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

***Knowledge and Understanding**- Lectures will systematically cover all the topics listed in the detailed syllabus below, dwelling on the most relevant and indispensable aspects, so as to provide students with the complete picture of the integrated topics and the correct study method to strengthen theoretical-practical knowledge*

***Applied Knowledge and Understanding skills** – Practical laboratory exercises and case studies will allow the students to learn the applications of the different methodologies, with a particular emphasis in protocol design and discussion of the different techniques*

***Autonomy of judgment** - the active learning methods implemented in this course will give the student the ability to design an experiment and develop ideas independently to solve a scientific question.*

***Communication skills** - active learning methods will be implemented to enable the student to acquire communication skills aimed at clearly presenting scientific work, communicating in appropriate technical language the methodologies learned and the results of their research.*

***Ability to learn** - the use of supplementary learning materials, including in the form of articles from the international scientific literature, will enable the student to continue studying mostly self-directed or independently.*

Where the continuation of the COVID-19 emergency makes it necessary to deliver distance learning activities, the course will be delivered via live streaming sessions on the telematics platforms available in the university (Microsoft Teams and Blackboard).

7. OTHER INFORMATIONS

Teachers will be available by appointment agreed by e-mail

8. METHODS FOR VERIFYING LEARNING AND FOR EVALUATION

The exam consists of the creation of a laboratory notebook collecting the protocols learned and the results obtained from the laboratory activity. The contents reported in the notebook will then be discussed in an oral interview in which the understanding of the methods carried out and the ability to apply them in the context of an experimental objective proposed by the lecturer will be

evaluated. The arguments of all four disciplines will be discussed in the oral examination. The test will be scored in 30s (minimum score: 18, maximum score: 30 cum laude). The examination grade will be the result of the weighted average of each of the four modules. Honors will be awarded, subject to the achievement of 30/30 in each module, to students who have demonstrated a superior level of subject knowledge and depth with autonomy of study, appropriateness of language and excellent communication skills.

The objective of the examination thus organized is to assess the student's acquisition of the following skills and knowledge:

*- **Knowledge and Understanding**- the appropriate level of knowledge of the topics covered in the program and the main methodologies acquired;*

*- **Applied Knowledge and Understanding skills** -The knowledge of scientific methodologies and their applications in relation to different biological problems;*

*- **Autonomy of judgment** - of the ability to develop an appropriate methodological approach in relation to different biological problems;*

*- **Communication skills** - of the appropriate language property and correct technical/scientific terminology;*

*- **Ability to learn** - of the ability to investigate and address issues of biological interest independently and through appropriate experimental approaches.*

Where the continuation of the COVID-19 emergency makes it necessary to conduct teaching activities remotely, learning assessments will be based on distance interview through use of the telematics platforms available in the university (Microsoft Teams and Blackboard).

9. PROGRAM

< The lab notebook>** How to record data and structure the notebook according to the type of experiment being conducted, what information to report, how to retrieve information from the notebook**.

< Advanced microscopy >

Atomic Force Microscopy, operating principle
Atomic Force Microscopy, Force-Distance curves acquisition and data analysis
Electronic microscopy
Confocal and multiphoton microscopy, advanced techniques: FRAP, FRET, FLIM
Analysis of FRAP, FRET, FLIM data using ImageJ and Microsoft Excel
Programming elements for image analysis: ImageJ Macro Language

< Proteomica e Metabolomica Clinica >

Proteomics and other post-genomic sciences

Sample preparation for protein expression analysis

Analysis of protein expression: electrophoretic and chromatographic techniques

Quantitative analysis

Statistics: software and website

Posttranslational modifications

Techniques used in proteomics and metabolomics

- *Electrophoretic techniques*
- *Chromatography*
- *Mass spectrometry*
- *NMR*
- *FT-IR*
- *Quantitative analysis by mass spectrometry*

< Isolation and characterization of stem cells >

Basic techniques of working under aseptic conditions.

Stem cell isolation techniques (hematopoietic stem cells and mesenchymal stromal cells).

Maintenance and expansion of cell cultures in adhesion and suspension.

Techniques for phenotypic characterization of primary cells in culture.

Protocols for induction of in vitro differentiation and morphological and molecular analysis of differentiation potential.

Secretome production and collection of mesenchymal stromal cells.

< Genomic medicine >

Genetic variant analysis by Sanger sequencing.

"In silico" analysis of genetic variants.

Databases for the interpretation of genetic variants.

Genomic analysis by "next generation sequencing" techniques: targeted gene sequencing panels, exome sequencing and transcriptomic analysis.

Gene expression analysis: RT-PCR, Real time PCR, Microarray.

Quantitative analysis of proteins: Western Blot.

Application of Southern Blot and RP-PCR for the detection of expanded alleles.

Laboratory practice: DNA extraction from peripheral blood, DNA sequencing, Western Blot.