UNIVERSITÀ DEGLI STUDI DI PALERMO

DEPARTMENT	Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche
ACADEMIC YEAR	2022/2023
MASTER'S DEGREE (MSC)	MOLECULAR AND HEALTH BIOLOGY
SUBJECT	BIOCHEMICAL METHODOLOGIES
TYPE OF EDUCATIONAL ACTIVITY	B
AMBIT	50507-Discipline del settore biomolecolare
CODE	05176
SCIENTIFIC SECTOR(S)	BIO/10
HEAD PROFESSOR(S)	ATTANZIO ALESSANDRO Professore Associato Univ. di PALERMO
OTHER PROFESSOR(S)	
CREDITS	6
INDIVIDUAL STUDY (Hrs)	98
COURSE ACTIVITY (Hrs)	52
PROPAEDEUTICAL SUBJECTS	
MUTUALIZATION	
YEAR	1
TERM (SEMESTER)	1° semester
ATTENDANCE	Mandatory
EVALUATION	Out of 30
TEACHER OFFICE HOURS	**ATTANZIO ALESSANDRO** Monday 11:00 13:00 Studio Docente, Dip. STEBICEF, via Archirafi 28, secondo piano.Per gli orari di ricevimento via Microsoft Teams si prega di fare rifermento a quelli in presenza. Tuesday 11:00 13:00 Studio Docente, Dip. STEBICEF, via Archirafi 28, secondo piano.Per gli orari di ricevimento via Microsoft Teams si prega di fare rifermento a quelli in presenza. Wednesday 11:00 13:00 Studio Docente, Dip. STEBICEF, via Archirafi 28, secondo piano.Per gli orari di ricevimento via Microsoft Teams si prega di fare rifermento a quelli in presenza. Thursday 11:00 13:00 Studio Docente, Dip. STEBICEF, via Archirafi 28, secondo piano.Per gli orari di ricevimento via Microsoft Teams si prega di fare rifermento a quelli in presenza.

DOCENTE: Prof. ALESSANDRO ATTANZIO

PREREQUISITES	Knowledges on the structure and function of biological macromolecules as well as the principal metabolic pathways acquired in the bachelor degree are requested. The possess of rudiments on basic laboratory methods is also considered an important prerequisite.
LEARNING OUTCOMES	KNOWLEDGE AND UNDERSTANDING ABILITY Ability of acquiring theoretical principles of advanced biochemical methods and evaluation of their impact on different areas of biochemical investigation. ABILITY TO APPLY KNOWLEDGE AND UNDERSTANDING - to individuate appropriate methodological approaches paying particular attention to recently introduced techniques and technologies; - to identify and independently manage a specific experimental design for basic and applied biochemical investigations. JUDGMENT SKILL The students have to be able: - to critically read a scientific manuscript evaluating the results in relationship to the applied methods; to make their own opinions on discussed topics and interdisciplinary develop the problem-solving skill proposing possible solutions for those aspects which are coherent with the proposed hypothesis. ABILITY TO COMMUNICATE INFORMATION The acquisition of communicative skills to spread basic knowledge with particular attention to the use of an appropriate technical-scientific language. In particular, the student should acquire: a) a certain degree of communicative ability, developing an efficacious language proficiency related to the scientific context; b) the ability to use adequate bioinformatic resources to introduce and discuss data in a scientific environment. LEARNING SKILL The students have to develop those learning skills necessary to consult bibliographic material, databanks and web sources. The ability either to undertake further studies on discussed topics with a high degree of autonomy or to transfer the acquired pieces of information in applied real situations.
ASSESSMENT METHODS	Student's evaluation will be performed by an oral exam and it will be expressed as out of 30. At least three questions will be required on the program course to ascertain whether the student has acquired an appropriate technical-scientific language, can critically discuss a scientific manuscript and independently propose an experimental design, evaluating the most appropriate methods for basic and applied biochemical assays. Evaluation will range between a minimum (18/30), whether the knowledge acquired by student is elementary and based on superficial factual knowledge, and a maximum (30/30 cum laude) whether the student provides evidence to have acquired mastery of methods for the biochemical investigation and their field of application.
EDUCATIONAL OBJECTIVES	The course wants to develop and deepen student's knowledge on biochemical methods applied to the study of proteins, their identification, protein-protein interactions and possible post-translational changes regulating their function. Particular attention will be also paid to the Real time PCR technique and its application in medicine and food-agricultural field. Aspects related to flow cytometry and its applications in the clinical biochemistry will be studied. The identification of cancer stem cells and their characterization will be also discussed.
TEACHING METHODS	The course is organized in lectures (40 h) and a laboratory training (12 h).
SUGGESTED BIBLIOGRAPHY	1. BIOCHIMICA E BIOLOGIA MOLECOLARE. PRINCIPI E TECNICHE. - WILSON KEITH-WALKER JOHN -RAFFAELLO CORTINA EDITORE. ISBN 978-88-3285-145-8 2. David L. Nelson Michael M. Cox I PRINCIPI DI BIOCHIMICA DI LEHNINGER (Settima edizione) ed. Zanichelli. ISBN 978-88-08-92069-0 3. Mauro Maccarrone. Metodologie biochimiche e biomolecolari. Zanichelli. ISBN 978-88-08-52055-5 Durante il corso verranno anche forniti articoli e monografie sugli argomenti svolti, nonché tutto il materiale informatico proposto.

SYLLABUS

Hrs	Frontal teaching
1	Introduction and aims of the course.
8	METHODS FOR THE STUDY OF PROTEINS. Introduction to proteomic approach for the study of proteins. Isolation and purification of proteins. Preparation of samples for proteomic analyses: solubilization, prefractioning and removal of contaminants. 2D-PAGE: Isoelectrofocusing (IEF). Choice of pH gradient. IPG strips. SDS-PAGE. Methods to identify proteins in the gel: Blue Comassie, Silver and SYPRO Ruby staining, potentialities and limitations of different dyes. Mass Spectrometry analysis: principles of mass spectrometry and anatomy of a mass spectrometer. Sources: Direct ionization, MALDI, ESI. Mass analyzers: Time of Flight (Tof), quadrupole, ion trap. Detectors. Peptide Mass FingerPrinting.

SYLLABUS

Hrs	Frontal teaching
4	METHODS FOR THE STUDY OF PROTEIN-PROTEIN INTERACTIONS. Role of protein-protein interactions and their classification. The origin of protein interactions and allostery in colocalization. Methods to study stable and transient interactions in proteins. Two hybrid assay. MAPPIT. Immunoprecipitation/Co-immunoprecipitation (CoIP). TAP tag method. Far western blotting. Protein arrays. Crosslinking. FRET.
8	QUANTITATIVE PCR. Introduction to Real time PCR. Fluorochrome chemistry. SYBR Green: advantages and limitations in Real Time PCR analyses. Melting curves for the detection of unspecific products. Fluorochrome-labelled probes: TaqMan probes, Molecular beacons, FRET probes, Scorpion primers, Eclipes probes, Lux primers, Plexor probes. Analysis of an amplification plot. Threshold cycle (Ct) in quantitative assessments. Optimizing a QPCR assay (regression line, correlation coefficient, amplification efficiency, use of replicates). Data analysis in Real Time PCR. Absolute quantification and standard curve. Relative quantification. Method of Ct and importance of the calibrators in QPCR assays. Use of housekeeping genes in real time PCR analyses. Livak method (2-DDCt) for quantitative relative analysis. Pfaffl method. Troubleshooting in real time PCR analyses.
7	ANTISENSE TECHNOLOGIES AND RNA INTERFERENCE TO STUDY RNA. Antisense RNA. First, second and third generation antisense oligonucleotides. Ribozymes. RNA interference and mechanism of action. Effects of dsRNA. Interferon response and Toll-like receptors. Tuschl roles for siRNA design. Silencing effects and methods to assess gene knockdown. Experimental controls in silencing assays. Factors affecting a reduced knockdown. Off-target effect. Strategies to reduce Off-target effect: concentration, pooling, base modifications. Mention on bioinformatics approaches. siRNA delivery. piRNA biogenesis and function. MicroRNA: biogenesis and processing. Microprocessor complex: Drosha and DGCR8. Argonaute proteins in RNA processing.
8	CYTOFLUORIMETRIC TECHNIQUES. General principles underlying flow cytometry. Sample preparation and fluorescent probes. Acquisition and analysis of the data obtained. Representation of flow cytometric data. Applications of flow cytometry: analysis cell surface antigens and intracellular biomarkers, evaluation of the cell cycle, study of apoptosis and other biochemical investigations.
4	METHODS TO STUDY CANCER STEM CELLS. Features of stem cells, classification and possible application in regenerative medicine. Cancer stem cells. Stem markers. Isolation and purification of cancer stem cells. Assays to study stem phenotype of cancer cells: side population, ALDH1, selection of surface markers, tumor spheres, assessment of invasive and tumorigenic potential.

Hrs	Practice
12	The pieces of information acquired during the course will be deepened by experimental approaches to provide an appropriate knowledge of methods and their applicability in routine laboratory investigations. Common methods for the study of proteins: purification, quantification and protein analysis. Flow cytometry and data analysis. The acquired information will be tested.