



UNIVERSITÀ
DEGLI STUDI DI MILANO-BICOCCA

SYLLABUS DEL CORSO

Introduzione alle Tecniche di Laboratorio

2021-2-E1301Q079

Aims

The aim of this course is to offer basic knowledge of the most common techniques of recombinant DNA and biochemistry. The course also aims to develop a critical sense in the choice of strategies for DNA cloning, production of recombinant proteins, purification of proteins, methods for quantification of proteins and enzymatic activity.

Knowledge and understanding - at the end of the course, students will know the theory underlying main biochemical laboratory techniques and basic techniques for the manipulation of recombinant DNA.

Ability to apply knowledge and understanding - at the end of the course students will be able to apply the knowledge acquired in choosing experimental approaches for DNA cloning, production, purification and characterization of proteins. This knowledge will also be applied in subsequent courses, in particular in practical course of integrated biology (Laboratorio integrato di biologia – LIB).

Autonomy of judgment - at the end of the course, students will be able to develop a protocol for cloning of plasmidic DNA, production of recombinant protein, protein purification and characterization.

Communication skills - at the end of the course, students are expected to acquire and to use adequate scientific terminology.

Learning skills - at the end of the course, students are expected to understand and critically evaluate the use of the methodologies reported in the scientific literature.

Contents

Recombinant DNA methods for cloning and producing recombinant proteins

Preparatory techniques for the extraction and enrichment of proteins

Preparatory techniques for protein purification

Electrophoretic and dosing techniques

Techniques for the analysis of the secondary and tertiary structure of proteins

Detailed program

Techniques of Recombinant DNA

DNA amplification (polymerase chain reaction)

DNA electrophoresis; restriction and ligation enzymes, choices of vectors and cloning hosts;

Methods for the production of recombinant proteins

Preparatory techniques for the extraction and enrichment of proteins

Cell lysis techniques; buffers and protein stability; salting out

Precipitation and fractionation in ammonium sulphate and dialysis

Centrifugative techniques (differential; in density gradient)

Preparatory techniques for protein purification

Chromatographies - general principles; filtration gel; ion exchange chromatography; chromatofocusing; reverse phase chromatography; hydrophobic chromatography; affinity chromatography

Strategies for protein purification

Electrophoretic and quantification techniques

Electrophoresis - general principles; SDS-PAGE; native electrophoresis; isoelectric focusing; two-dimensional electrophoresis; western blotting

UV-VIS spectroscopy

Quantification of protein concentration and enzymatic activity

Techniques for the analysis of the secondary and tertiary structure of proteins

Circular dichroism

Spectrofluorimetry

Mass spectrometry

Prerequisites

A basic knowledge of general and organic chemistry, physics and biochemistry is essential. The essential physico-chemical principles and biochemistry topics will be reviewed

Teaching form

Classroom lectures based on powerpoint presentations; videos; active learning (discussion of experimental data)

Textbook and teaching resource

PPT slides, published on the Moodle website (<http://elearning.unimib.it/>).

Textbooks:

- K. Wilson & J. Walker (2010). Principles and Techniques of Biochemistry and Molecular Biology.
- M. C. Bonaccorsi di Patti, R. Contestabile, M. L. Di Salvo "Metodologie Biochimiche" Casa Editrice Ambrosiana, 2012

Semester

Second semester

Assessment method

Written + oral exam. Written exam (90 min) consists in 10 questions / exercises on theoretical topics and on experimental data obtained with the techniques covered by this course. Oral exam (approx. 15 min) consists of one - two questions that, in most cases, are inspired by the written exam. The exam is aimed at evaluating the knowledge acquired in the theoretical field and in the interpretation of experimental data. The linguistic capacities are also assessed, as for the use of scientifically and technically appropriate language. The final grade is obtained by the average of the two marks.

Office hours

On demand, by mail to stefania.brocca@unimib.it
