UNIVERSITÀ
DEGLI STUDI DI MILANO-BICOCCA

SYLLABUS DEL CORSO

Introduzione alle Tecniche di Laboratorio

2122-2-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

1. Recombinant DNA methods for cloning and production of recombinant proteins

2. Preparative techniques for protein extraction and enrichment

3. Techniques for assaying protein and enzyme activity

4. Preparative techniques for protein purification

5. Electrophoretic and immunochemistry techniques

6. Biophysical techniques for conformational analysis of proteins

Detailed program

1. Recombinant DNA methods for cloning and production of recombinant proteins. Methods for the production of recombinant proteins; Restriction and ligation enzymes; Choices of cloning vectors and hosts; DNA amplification (polymerase chain reaction); Plasmid DNA extraction methods; DNA electrophoresis; Introduction to sequencing techniques.

2. Preparative techniques for protein extraction and enrichment. Centrifugation and ultrafiltration techniques; Ammonium sulfate precipitation and fractionation; Cell lysis and fractional centrifugation techniques.

3. Techniques for protein and enzyme activity assay. Protein concentration assay; Enzyme activity' assays; Purification table.

4. Preparative techniques for protein purification. Introduction to chromatographic techniques; Introduction to chromatographic techniques; Molecular exclusion chromatography; Ion exchange chromatography; Hydrophobic interaction and reverse phase chromatography; Affinity chromatography; HPLC and FPLC; Evaluating the progress of a purification.

5. Electrophoretic and immunochemical techniques. Electrophoresis in native and denaturing conditions (SDS-PAGE); Isoelectrophoresis (IEF) and two-dimensional electrophoresis (2D-PAGE); Western blotting; Introduction to immunochemical techniques; Immunoprecipitation, ELISA

6. Biophysical techniques for protein conformational analysis. Mass spectrometry; Introduction to UV-vis spectrophotometry and absorption; Circular dichroism spectroscopy; Spectrofluorimetry; Fluorescence resonance energy transfer (FRET) techniques.

Prerequisites

Knowledge of basic concepts of physics and general and organic chemistry is required. Most relevant physicochemical principles and biochemistry concepts will be briefly stated or recalled at the beginning of class.

Teaching form

Classroom lectures based on powerpoint presentations; videos; 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

First semester

Assessment method

Written + oral exam. Written exam (90 min): consists of 10 multiple choice questions, 2 exercises and 2 open-endend questions. Oral exam (approx. 15 min): consists of one-two questions that, in most cases, are inspired by the written exam. Through both tests will be evaluated the knowledge acquired in the theoretical field and in the interpretation of experimental data. The expositive abilities are also evaluated, based on their coherence and the use of a scientifically and technically appropriate language. The grade of both tests, written and oral, is assigned in thirtieths. The final grade is obtained from the average of the two marks.

Office hours

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