UNIVERSITÀ
DEGLI STUDI DI MILANO-BICOCCA

COURSE SYLLABUS

Introduction To Laboratory Techniques

2324-2-E1301Q079

Aims

The aim of this course is to provide students with a basic knowledge of the most common techniques of biochemistry and molecular biology, aimed at the production of recombinant proteins. The course also aims at describing the complementarity of cloning strategies, production of recombinant proteins, protein purification, and their structural and functional characterisation, the latter referring mainly to 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 should be able to understand and recognise the methodologies learnt in different contexts (e.g. in scientific articles).
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Contents

1. Preparative techniques for protein extraction and enrichment

2. Electrophoretic and immunochemistry techniques

3. Techniques for assaying protein and enzyme activity

4). Preparative techniques for protein purification

5. Biophysical techniques for conformational analysis of proteins

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

Detailed program

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

2. 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

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

6. 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.

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; description and interpretation 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 examination. 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. Both examinations aim to assess the theoretical knowledge, skill acquired in the interpretation of experimental data, and the understanding of complementary relationships between different techniques and experimental approaches. The expositive abilities are also evaluated, based on their coherence and the use of a scientifically and technically appropriate language. The grade of the written test and the oral interview 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

Sustainable Development Goals

QUALITY EDUCATION