

Integrated Laboratory Class 5

Code: 100882
ECTS Credits: 3

Degree	Type	Year	Semester
2500252 Biochemistry	OB	3	1

Contact

Name: Mohammed Moussaoui
Email: Mohammed.Moussaoui@uab.cat

Use of languages

Principal working language: catalan (cat)
Some groups entirely in English: No
Some groups entirely in Catalan: Yes
Some groups entirely in Spanish: No

Prerequisites

Student must be attending simultaneously or have taken the theory subjects corresponding to the contents of the laboratory practices.

In order to be able to attend the practices, the student must justify having passed the biosafety and security tests that he will find on the Virtual Campus and be knowledgeable and accept the rules of operation of the laboratories of the Faculty of Biosciences.

Students are advised to review the theoretical contents on which this subject is based

Objectives and Contextualisation

The subject of Integrated Laboratory V is part of a set of six subjects that are distributed throughout the first six semesters of the Degree in Biochemistry.

The objective of these subjects is the acquisition of practical skills of the student.

The contents are organized in an increasing order of complexity, associated with the needs and acquisition of theoretical contents.

During the Integrated Laboratory V the student acquires practical competences in the following contents:

- Recombinant DNA
- Immunology
- Cell signaling
- Industrial Biochemistry
- Proteomics

The practices in the laboratory focus on the learning of basic techniques and specific to each field and on the own characteristics of working in the laboratory.

Content

- **Module of Recombinant DNA and Proteomics: 8 sessions of 4 hours**

A) Design of primers and PCR.

B) Purification and digestion of PCR product. Insertion by ligation in a vector of expression and transformation of E.coli competent cells.

C) Extraction by Minipreparation of plasmid DNA and analysis by electrophoresis of the products of digestion of cloning.

D) Obtaining of the cell extract and purification of the dhfr protein by column of affinity to the histidines tagged protein.

E) Electrophoresis on PAGE-SDS. Determination of the amount of protein and enzymatic activities. Evaluation by calculation of the yield and the purification factor of the purification process.

F) Visit to the proteomics service. Characterization of the purified dhfr by mass spectrometry. Analysis and sequencing of the peptides purified by mass spectrometry through the MS / MS technique and identification of unknown proteins present in the sample by peptide mass fingerprinting. Use of similarity databases and search programs

G) Obtaining of lysozyme crystals by hanging drop method. Visualization and manipulation of crystals obtained.

I) Oral presentation of the results obtained and open turn of questions.

• **Module of Bioreactors Biochemistry: 2 sessions of 4 hours**

A) Learning the operation and the main characteristics of a bioreactor.

B) Application of this knowledge in the bioreactor expression process of the dhFR protein in E. coli.

C) Monitoring the growth of the crop in fermenter.

• **Module of Immunology: 3 sessions of 4 hours**

A) Separation of splenocytes and cell count

B) Precipitation of human Igs and dialysis. Quantification of Igs in human serum (ELISA)

C) Analysis of the functional level of the complement in a sample of human serum. Calculation of the CH50