Integrated Laboratory Class 4

Code: 100925
ECTS Credits: 3

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Contact
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Email: Sandra.Villegas@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

Teachers
Francesc Gòdia Casablancas
Regina Martínez Barchino
Jesús Aranda Rodríguez
Maria del Mar Marquès Bueno

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

You must have passed the safety test in laboratories. The test is answered in the corresponding space of the Virtual Campus and the information that must be consulted is in the space for communication of the Degree in Biotechnology.

Objectives and Contextualisation
The Integrated Laboratory 4 is the fourth subject of a set of 6 that are distributed over the 6 semesters corresponding to the first three years of the Degree in Biotechnology.

The training objectives of these subjects focus on the acquisition of competences within the framework of the practical training of the student.

The contents are organized in a growing order of complexity and associated to the needs and progress of the theoretical contents of the Degree.

The Integrated Laboratory 4 has as its training objectives the acquisition of practical skills in 4 modules:
- Biology and Molecular Genetics, and Recombinant DNA Techniques
- Molecular Microbiology
- Bioreactors
- Numerical Methods and Computer Applications.

These modules are grouped into two blocks:

1- Manipulation of organisms: The foundations of Molecular Biology and Genetics are necessary for the understanding of Recombinant DNA Techniques, while working on the mechanisms of the transmission of genetic information between microorganisms and their modification in the laboratory.

2- Fundamentals for the design of bioreactors and development of bioprocesses: The aim is to acquire basic knowledge in the design, operation and characterization of the main types of bioreactors, and the approach and resolution of the mathematical equations derived from them.

**Competences**

- Apply general laboratory security and operational standards and specific regulations for the manipulation of different biological systems.
- Apply the principal techniques for the use of biological systems: recombinant DNA and cloning, cell cultures, manipulation of viruses, bacteria and animal and plant cells, immunological techniques, microscopy techniques, recombinant proteins and methods of separation and characterisation of biomolecules.
- Describe the principles behind the design and functioning of bioreactors and calculate, interpret and rationalise the main parameters in transport phenomena and the matter and energy balances in biotechnological processes.
- Interpret experimental results and identify consistent and inconsistent elements.
- Lead and manage teams, and develop capacities for organisation and planning.
- Make decisions.
- Search for, obtain and interpret information from the principal databases on biology, bibliography and patents and use basic bioinformatic tools.
- Think in an integrated manner and approach problems from different perspectives.
- Use ICT for communication, information searching, data processing and calculations.
- Use the fundamental principles of mathematics, physics and chemistry to understand, develop and evaluate a biotechnological process.
- Work individually and in teams

**Learning Outcomes**

1. Apply the different waste disposal processes correctly.
2. Apply the fundamental techniques used in the analysis, purification, and characterisation of biomolecules.
3. Apply the general safety rules in place in a biotechnology laboratory.
4. Apply the principles of sterility to processes of manipulation and counting of microorganisms.
5. Describe the theoretical grounding and apply the appropriate techniques for the structural and functional characterisation of proteins and nucleic acids.
6. Extract complementary information from databases to support the analysis of results and the writing of reports on experiments.
7. Interpret experimental results and identify consistent and inconsistent elements.
8. Lead and manage teams, and develop capacities for organisation and planning.
9. Make decisions.
10. Obtain significant experimental data to calculate transport phenomena and balances of matter and energy.
11. Think in an integrated manner and approach problems from different perspectives.
12. Use ICT for communication, information searching, data processing and calculations.
13. Use the basic techniques for handling, separating, detecting and analysing proteins and nucleic acids.
14. Use the basic techniques for studying biomolecules in a chemistry laboratory.
15. Use the techniques for cultivating prokaryote and eukaryote cells and for manipulating biological systems.
16. Work individually and in teams
Content

The subject is structured in 4 modules.

Biology and Molecular Genetics, and Recombinant DNA Techniques

The practices of this module will be carried out during 5 sessions (four of 3.5 hours + one of 3 hours).

Practice 1 (1-5 sessions). Basic concepts of DNA cloning

The objective of the practice is to present in an integrated way, by means of a simple experimental model, some of the basic stages and methodologies underlying the cloning of DNA: preparation of E. Coli competent cells, transformation with a mixture of plasmids, selection of transformants, screening of phenotypic characteristics by replica in specific media plates, culture in liquid medium and plasmid DNA extraction (pDNA), digestion of pDNA with restriction enzymes and analysis by electrophoresis Agarose gel (which allows to establish the correlation between phenotype and genotype). This practice will be extended continuously from the first to the fifth session according to the following schedule:

1st session. Preparation of competent cells and transformation.

2nd session. Replica of transformer colonies in plate and inoculation in liquid medium.

3rd session. Minipreparation of plasmid DNA from transformants grown in the liquid medium. Read the replicas on the plate.


5th session. Electrophoresis on agarose gel. Evaluation of the results.

Practice 2 (3h). Extraction and spectrophotometric analysis of genomic DNA

A genomic DNA preparation will be made from E. Coli cells (alternatively, from rat liver), and the absorption spectrum will be obtained in the ultraviolet region. With the measurements of absorbance at 260, 280 and 230 nm, the purity of the obtained preparation will be quantified and determined. The hyperchromic effect for denatured DNA will be studied.

1st session. Extraction of genomic DNA.

4th session. Spectrophotometric analysis.

5th session. Evaluation of the results.

Practice 3 (3h). Superhelicity of DNA

The topology of a pDNA will be analyzed by means of a kinetic test with topoisomerase I. The reactions will be checked by agarose gel electrophoresis.


3rd session. Electrophoresis on agarose gel.

5th session. Evaluation of the results.

Molecular Microbiology

The Molecular Microbiology module is organized into 5 sessions. The practices in these sessions will allow the student to learn the basic techniques of DNA transfer in bacteria, the mechanisms of directed and random mutagenesis used for the genetic modification of prokaryotes, and the mechanisms that allow the study of gene expression and its regulation in bacteria. All these contents will be grouped into the 4 practices that are listed below.
Practice 1 (4h) Transfer of genetic material into prokaryotes

Different methodologies will be used for the incorporation of exogenous DNA into bacteria, such as transformation mechanisms, biparental conjugation, triparental conjugation, and transduction of markers between bacteria.

Practice 2 (2h). Processes of mutagenesis and recombination to obtain new strains

Basic processes for the interchange of bacterial genetic material will be applied, such as experiments to obtain spontaneous mutants, directed mutagenesis, or the integration and/or replacement of genetic material by recombination.

Practice 3 (4h). Use of mobile genetic elements to obtain mutants

Methodologies based on the use of mobile genetic elements for bacterial genetic manipulation will be used. The type of jumps of these elements will be described, as well as their frequency of movement.

Practice 4 (2h). Control of gene expression in prokaryotes

The tools for the quantification of the bacterial gene expression will be applied, and these methodologies will be used to study regulated promoters as well as to identify the mechanisms that control their gene expression.

Bioreactors

The practices are organized in 4 sessions of 3 h.

Practice 1 (3h) + Practice 2 (3h). Continuous Stirred Tank Reactor (RCTA)

The operation and the main characteristics of an RCTA type bioreactor are learned. The kinetics of growth of a yeast strain are determined. The stimulus-response techniques are used to determine the distribution of the residence time of the bioreactor, and analyze its hydrodynamic behavior, in particular the mixing characteristics. All this knowledge is included in the equations for the design of RCTA-type bioreactors.

Practice 3 (3h) + Practice 4 (3h). Air-lift reactor.

The operating bases of an Air-lift bioreactor are learned, as well as the different elements involved in its design. The experimental techniques to determine the coefficient of oxygen transfer between a gas phase and a liquid, kLa are used. The influence of the operating conditions of the bioreactor on the properties of gas-liquid transference is studied.

The methodology is analyzed to determine the oxygen consumption of a yeast culture.

Numerical Methods and Computer Applications

They are organized in 5 sessions of two and a half hours that are done in the computer room.

Practice 1 (2.5h) Introduction.

The objective is that the student becomes familiar with the programming environment that will be used in these practices. You will see the basic instructions and instructions for the programming of algorithms.

Practice 2 (2.5h) Errors.

The purpose of this practice is to know the limitations of numerical errors. We will see how to detect and control different sources of error in the scientific calculation.

Practice 3 (2.5h) Function Zeros.

In this practice, different numerical methods will be implemented for the calculation of zeros of functions. Its applicability will be studied in different cases.
Practice 4 (2.5h) Integration.

In this practice, polynomial interpolation algorithms will be developed and different numerical methods will be implemented to evaluate defined integrals.

Practice 5 (2.5h) Differential equations.

The objective of this practice is to implement some basic numerical resolution methods for simple cases. You will also see how to use software routines based on more advanced methods.

**Methodology**

The attendance to the classes of this subject is obligatory since they imply an acquisition of competitions based on the practical work.

Biology and Molecular Genetics, Recombinant DNA Techniques, Molecular Microbiology and Bioreactors: Practical classes of laboratory and data analysis.

The students carry out the experimental work in groups of 2 and under the supervision of the responsible professor.

The practical protocols and, if applicable, the questionnaires for response, will be available on the Virtual Campus of the subject.

Before beginning a practical session the student must have read the protocol and know therefore the objectives of the practice, the foundations and the procedures that must be carried out.

If so, you must know the specific safety and waste treatment measures.

When completing the practice of the module of Bioreactors, the students will have to work with the obtained data and present the corresponding reports.

In the practical sessions you have to take:

- Protocol and, if applicable, the questionnaire.
- A notebook to collect the information of the experimental work.
- Laboratory baton.
- Safety glasses.
- Permanent marker

Numerical Methods and Computer Applications: Practical classes in the computer rooms of the faculty.

The students will carry out the proposed work in the practice script under the supervision and direction of the responsible professor. In each session the student will complete a questionnaire on the different problems resolved in practice.

The practical scripts will be available on the Virtual Campus of the subject.

Before beginning a practical session the student must have read the script and know therefore the objectives of the practice and the foundations of the numerical methods that he will have to use.

In the practical sessions you have to take:

- The script of the practice.

**Activities**

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Assessment

Attendance at practical sessions is mandatory. The students will obtain the "Non-Avaluable" qualification when the absence exceeds 20% of the programmed sessions.

Students who do not obtain the minimum qualification of 4, required to be able to pass each one of the modules of the integrated laboratory, will not pass the subject. In this case, the final maximum grade of the subject will be 4.

Due to the fact that the Integrated Laboratory is differentiated in modules, from the second enrollment, repeat students will only have to evaluate the specific modules that have not been exceeded. This exemption will be maintained for a period of three additional tuition fees, participating in a number of assessment activities that can not be granted, at best, the qualification of approved.

The evaluation of each module will be carried out independently, following the criteria detailed below.

The final evaluation of the subject will be obtained from the average assessment of the different modules (33.34%, 22.22%, 22.22%, 22.22%).

Biology and Molecular Genetics, and Recombinant DNA Techniques
This module will be evaluated by means of an individual examination in which questions and exercises related to the practices will have to be solved.

The exam will take place once the practice sessions of the four groups have finished (see the calendar).

The supervision of the work and the results obtained in the laboratory, which will be carried out during the realization of the practices (continuous assessment), will provide up to 10% of the module's note.

Molecular Microbiology
Two different aspects will be taken into account, on the one hand, the mark obtained in a questionnaire that will be done at the end of the session 5 and which will refer to all the practices that make up this module, and on the other, the achievement of the Targets set in each of the programmed practices. The questionnaire will represent 70% of the final grade of the module while the remaining 30% will depend on the evaluation of the results obtained and the experimental work performed.

Bioreactors
Two different aspects will be taken into account: the quality of work in the laboratory and the obtained experimental data (50%) and the elaboration of the report of the practices (50%), including the questions that are proposed in the same.

The reports must be submitted before a specific date, which will be announced at the beginning of the laboratory. The delay not justified in the presentation of the reports will imply a punishment in the punctuation of the same.

Numerical Methods and Computer Applications
This module will be evaluated by means of an individual examination at the end of the semester, which will mean 70% of the final mark, and the questionnaires that must be submitted at the end of each practice and which will represent the remaining 30%. In the final exam the student will have to solve some problems similar to those that have been dealt with in the practices.

Assessment Activities
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**Bibliography**

Bibliography and web links are indicated in the practices protocols or, where appropriate, in the Teaching Guide of the corresponding theory subject.