Universitat Autònoma de Barcelona

2018/2019

Integrated Laboratory Class 4

Code: 100925
ECTS Credits: 3

Degree	Type	Year	Semester
2500253 Biotechnology	OB	2	2

Contact

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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

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Pablo Fernandez Millan
Jesús Aranda Rodríguez

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.

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.

4th session. Plasmid DNA digestion with restriction enzymes. Agarose gel preparation.

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.

2nd session. Assay with Topoisomerase I. Agarose gel preparation.

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 ofjumps 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 toidentify 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 gasphase and a liquid, kLa are used. The influence of the operating conditions of the bioreactor on the properties of gas-liquid transfrence 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.