

**Integrated Laboratory Class 5**

Code: 100924  
ECTS Credits: 3

Degree	Type	Year	Semester
2500253 Biotechnology	OB	3	1

### 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

Pau Ferrer Alegre

### Prerequisites

It is recommended that students are taking or have already completed the theoretical subjects related to the contents of this lab: Recombinant DNA Technology, Chemistry and Protein Engineering, Process Engineering Fundamentals, Bioreactors, and Separation and Purification processes.

To attend the sessions, the student must have passed the Biosafety and Security tests found in Campus Virtual. In addition, the student should be aware and accept the functioning guidelines of the laboratories of the Faculty of Biosciences.

### Objectives and Contextualisation

This practical course integrates key concepts and techniques from different fields of biotechnology. In particular, this practical course combines topics from the recombinant DNA technology, protein chemistry and bioengineering (including the growth of microorganisms in bioreactors, and recovery and purification of proteins) in the designing and development of a biotechnological process. In this way, the overall objective of this course is to obtain an overview of three of the most important parts of biotechnological processes and has the following specific objectives:

- Learn the different stages which comprise the extraction of total RNA from eukaryotic tissues, as well as the key points of the reaction of RT-PCR to amplify the specific sequences of cDNA including the basic criteria used in the designing oligonucleotides for RT-PCR.
- Know the main features of the expression vectors used for the production of recombinant proteins.
- Know the different steps of standard molecular cloning process consisting in: digestion with restriction enzymes, ligation reaction and transformation in a bacterial host, as well as the criteria used for the recognition of clones bearing vector and foreign DNA.
- Know the key parameters for heterologous expression of proteins in bacteria, as well as to design experiments of recombinant expression according to the characteristics of the protein to be expressed.

- Design and apply protocols for the purification of recombinant proteins.
  - Characterize expressed proteins both at structural and functional levels
  - Learn the basic sequence of operations, their distribution over time, and the key points associated with the growth of microorganisms in bioreactors and the purification of recombinant proteins at the laboratory scale.
  - See the interactions between the different stages of a process of production of recombinant proteins, the characteristics of the protein to be produced, the expression system used in the fermentation process, and the recovery and purification of the recombinant protein .
  - Characterize at the laboratory scale the key parameters of the production process -growth in bioreactor, recovery and purification- of the recombinant protein of interest.
- Integrate all the results obtained, evaluate the production system of the recombinant protein globally and discuss and design strategies for optimization.

## Competences

- Act with ethical responsibility and respect for fundamental rights and duties, diversity and democratic values.
- 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 bioindustrial processes.
- Design and implement a complete protocol for obtaining and purifying a biotechnological product.
- Design continuation experiments for problem solving.
- Interpret experimental results and identify consistent and inconsistent elements.
- Lead and manage teams, and develop capacities for organisation and planning
- Learn new knowledge and techniques autonomously.
- Make decisions.
- Obtain information from databases and use the software necessary to establish correlations between the structure, function and evolution of macromolecules.
- Search for, obtain and interpret information from the principal databases on biology, bibliography and patents and use basic bioinformatic tools.
- Take account of social, economic and environmental impacts when operating within one's own area of knowledge.
- Take sex- or gender-based inequalities into consideration when operating within one's own area of knowledge.
- Think in an integrated manner and approach problems from different perspectives.
- Use ICT for communication, information searching, data processing and calculations.
- Use analytical methodologies for assaying the biological activity of cellular components, especially enzymes, both in vitro and in vivo.
- 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. Act with ethical responsibility and respect for fundamental rights and duties, diversity and democratic values.
2. Apply the different waste disposal processes correctly.
3. Apply the fundamental techniques used in the analysis, purification, and characterisation of biomolecules.
4. Apply the general safety rules in place in a a biotechnology laboratory.
5. Apply the principles of sterility to processes of manipulation and counting of microorganisms.

6. Describe the fundamental theory behind the basic and advanced techniques for obtaining and characterising biomolecules.
7. Describe the theoretical grounding and apply the appropriate techniques for the structural and functional characterisation of proteins and nucleic acids.
8. Design continuation experiments for problem solving.
9. Execute in the laboratory protocols for obtaining and purifying industrial products with applications in biotechnology.
10. Extract complementary information from databases to support the analysis of results and the writing of reports on experiments.
11. Interpret experimental results and identify consistent and inconsistent elements.
12. Lead and manage teams, and develop capacities for organisation and planning
13. Learn new knowledge and techniques autonomously.
14. Make decisions.
15. Obtain significant experimental data to calculate transport phenomena and balances of matter and energy.
16. Propose strategies for purifying complex-mixture biomolecules.
17. Take account of social, economic and environmental impacts when operating within one's own area of knowledge.
18. Take sex- or gender-based inequalities into consideration when operating within one's own area of knowledge.
19. Think in an integrated manner and approach problems from different perspectives.
20. Use ICT for communication, information searching, data processing and calculations.
21. Use ICT tools to compare sequences and calculate kinetic parameters.
22. Use the appropriate methodology for studying the different types of biological samples.
23. Use the basic computer tools for calculating kinetic parameters.
24. Use the basic techniques for analysing enzyme activity.
25. Use the basic techniques for handling, separating, detecting and analysing proteins and nucleic acids.
26. Use the basic techniques for studying biomolecules in a chemistry laboratory.
27. Use the instrumentation necessary for the different techniques for separating and characterising biomolecules.
28. Use the techniques for cultivating prokaryote and eukaryote cells and for manipulating biological systems.
29. Work individually and in teams

## Content

This practical course is carried out entirely in the laboratory and includes fourteen sessions.

- Session 1: Introduction. Review of the biotechnological methods used in this course. General characteristics of the cloning vectors for the expression of recombinant proteins. Using databases to fetch the coding sequence of the human dihydrofolate reductase (hDHFR) cDNA. Designing the primers to amplify the hDHFR cDNA by RT-PCR.
- Session 2: Obtaining the coding sequence of DHFR gene: Purification of total RNA from a small sample of human blood. Reverse transcription using polyA as primer. PCR amplification of the cDNA from hDHFR gene.
- Session 3: Cloning of the DHFR gene into a prokaryotic expression vector (I) and recombinant DHFR purification. Purification of PCR products. Preparing the ends of insert and cloning vector. Inactivation of restriction enzymes. Ligation reaction. Strategies to maximize the ligation of the insert to cloning vectors. Preparing *E. coli* cultures for production of recombinant proteins in a small scale. Induction of the expression of the recombinant hDHFR.
- Session 4: Cloning of the DHFR gene into a prokaryotic expression vector (II) and recombinant DHFR purification (I): *E. coli* transformation with the ligation obtained at the end of session 3. Affinity

chromatography column setup. Obtaining a cell extract and isolation of soluble and insoluble fractions. Purification by affinity chromatography of recombinant hDHFR. Interpreting the chromatographic profile obtained and identifying the samples of interest.

- Session 5: Recombinant DHFR purification (II) and identification of the clones bearing the recombinant vector (I): Analysis of the affinity purified hDHFR samples by SDS-PAGE. Enzyme activity characterization. Purification table. Colony selection and preparation of small scale *E. coli* cultures.
- Session 6: Characterization of recombinant DHFR (I) and identification of the clones bearing the recombinant vector (II): Confirming the identity of hDHFR by mass spectrometry fingerprint (i). Determining the molar extinction coefficient (molar absorptivity) of hDHFR. Isolation of plasmid DNA from *E. coli* cultures. Digestion plasmid DNA with restriction enzymes.
- Session 7: Characterization of recombinant DHFR (II) and identification of the clones bearing the recombinant vector (III): Confirming the identity of hDHFR by mass spectrometry fingerprint (ii). Molecular mass of the hDHFR by MALDI-TOFF. General discussion of the results obtained in the hDHFR purification and characterization experiments. Electrophoresis of the DNA samples and identification of the positive clones. General discussion of the results obtained in the cloning procedure.
- Session 8: Designing a process to produce recombinant proteins: Bioreactor preparation and operation. Preparation of starter cultures. Experimental design proposal as a tool to improve and redesign the production process.
- Session 9: Bioreactor culture (I): Bioreactor inoculation and checking the culture parameters (temperature, pH,  $pO_2$ , cell mass, substrates subproducts, plasmid stability and hDHFR product) when operating in batch. Calculation of growing velocity, substrate utilization, subproduct production, yields and productivity. Inducing recombinant hDHFR expression.
- Session 10: Bioreactor culture (II) and the DHFR recovery (I): Bioreactor stop and cell mass recovery using centrifugation. Cleaning bioreactors.
- Session 11: DHFR recovery (II): Selection of a recovery system: implications and consequences of the selection steps when recovering and purifying the recombinant protein. Cell disruption by enzymatic and mechanical procedures. Recovery of the soluble fraction by centrifugation and microfiltering.
- Session 12: DHFR purification (I): Preparative purification of recombinant hDHFR from the cell mass by affinity chromatography. Recovery of the different chromatographic fractions.
- Session 13: DHFR purification (II): Analysis of the chromatographic purification (total protein by Bradford, purification level by SDS-PAGE and enzyme activity of hDHFR purity and activity). Consistency of the results. Performance, purification tables and purification factor.
- Session 14: Global analysis of the results. Evaluation of experimental data. Obtaining information to improve and redesign the production process

## Methodology

Since the objective of this course is the acquisition of practical skills, the attendance to all lab sessions of this course is mandatory.

For the realization of the practical activities, the students are distributed in four turns of approximately 20 students. For the experimental sessions of parts A and B, students will perform the experimental work in groups of two people. For the experimental sessions of parts C and D students will perform the experimental in groups of four-five people. For all the parts will be programmed sessions of introduction and discussion of results that will held also in the labroom with all the students of each turn. All the experimental sessions will be supervised by one or two instructors.

Students will be accommodated in groups of two people in parts A and B. In parts C and D the students will be distributed in groups of four people. All practice sessions will be supervised by one or two instructors.

The experimental recipes or protocols and all the information necessary for the perform the lab sessions will be available in advance in Campus Virtual. Before starting each lab session is highly recommended that students have carefully reviewed all the written material supplied. In addition, the student should also be aware of the laboratory safety measures and should also know the different types of waste disposal procedures.

In the event that this subject is surveyed and the period for answering surveys coincides with a lab session, students will have fifteen minutes to answer the teacher evaluation survey.

In each lab session all the students must bring a:

- Hard copy of the experimental protocols.
- Lab notebook.
- Lab coat.
- Safety goggles.
- Permanent marker.

Annotation: Within the schedule set by the centre or degree programme, 15 minutes of one class will be reserved for students to evaluate their lecturers and their courses or modules through questionnaires.

## Activities

Title	Hours	ECTS	Learning Outcomes
Type: Directed			
Lab classrooms	56	2.24	2, 5, 20, 4, 3, 7, 6, 8, 9, 10, 11, 12, 15, 19, 14, 16, 29, 27, 22, 23, 21, 24, 26, 25, 28
Type: Supervised			
Tutorials	3	0.12	20, 8, 11, 12, 19, 14, 16, 29, 23, 21
Type: Autonomous			
Study	6	0.24	13, 20, 6, 8, 10, 11, 19, 14, 16, 23, 21
Writing lab reports	6	0.24	13, 20, 7, 6, 8, 10, 11, 12, 19, 14, 16, 29, 23, 21, 24

## Assessment

The subject will be assessed taking into account the marks obtained by the student in two exams, in the different lab reports written at the end of the lab sessions and finally in an appreciation by the different teachers of the behavior, performance and work of each student during the lab sessions. The weight of each of these parts is the following:

- Written exam corresponding to sessions 1-7: 30%
- Written exam corresponding to sessions 8-14: 25%

- Report of sessions 1-7: 15%. This report will consist of two parts. The first one will basically deal with the issues related to recombinant DNA technology and the second one in which preferentially the topics related to chemistry and protein engineering will be discussed.
- Report of the sessions 8-14: 20%
- Assessment of attitude and work: 10%

For sessions 1 to 7, the assessment of the behavior, performance and work will be based on the following rubrics: a) Punctuality, 20%; b) Answer appropriately questions about the lab script, 20%; c) Attention during the explanations of the teacher, 20%; d) Equitable participation in the work of the different students of each group, 20%; d) Participation in the interpretation and discussion of the results, 20%.

Failure to attend a practice session will lead to a 50% reduction in the average score of the reports when no justified. In case of not attending more than one practical session without justification, this note will be reduced to 0.

As it is a practical subject, it is necessary to attend at least 80% of the scheduled sessions (11 sessions) in order to pass the subject, regardless of whether the absences are justified or not. To pass the subject, the student must also obtain a final grade equal to or greater than 5.

A student will obtain the final grade of "No Avaluable" when he has attended less than 50% of the lab sessions or when, even obtaining the maximum grade in all the activities carried out, he doesnot reach the global grade of 5. Repeating students should only do (and be assessed) about the topics that they previously failed (grade lower than 5) in the first enrollment. Grades will be saved for a maximum period of two additional enrollments of the subject.

The reviewing of each one the assessment results will be announced within 10 working days following the publication of the assessment results. If the student does not attend this review session, this assessment will not be reviewed later.

Grade with Honors (MH) is the decision of the faculty. The regulations of the UAB indicate that MH can only be awarded to students who have obtained a final grade of 9.0 or more. Grade with honors can only be granted up to 5% of of the total number of students enrolled in the subject.

Notwithstanding other disciplinary measures deemed appropriate, the irregularities committed by the student that can lead to a variation in the rating of an assessment will be graded with a zero. Therefore, copying, plagiarizing, cheating, copying, etc. In any of the assessment activities it will imply a grade of 0 (zero).

## Assessment Activities

Title	Weighting	Hours	ECTS	Learning Outcomes
Attitude and quality of work in the laboratory	10%	0	0	1, 18, 17, 2, 4, 12, 19, 14, 29, 26
Written exam corresponding to sessions 1 to 7	30%	2	0.08	5, 3, 6, 8, 11, 19, 14, 16, 27, 22, 24, 25, 28
Written exam corresponding to sessions 8 to 14	25%	2	0.08	5, 3, 6, 8, 9, 11, 15, 19, 14, 16, 27, 28
Written lab report corresponding to sessions 1 to 7	15%	0	0	13, 20, 7, 6, 8, 10, 11, 12, 19, 14, 16, 29, 23, 21, 24
Written lab report corresponding to sessions 8 to 14	20%	0	0	13, 20, 7, 6, 8, 10, 11, 12, 19, 14, 16, 29, 23, 21, 24

## Bibliography

The next references are freely available as PDF documents in the Campus Virtual of the subject:

- Affinity Chromatography. Principles and Methods. Amersham

- pET system manual. Novagene

- Eiteman, Altman (2006) Overcoming acetate in Escherichia coli recombinant protein fermentations. Trends in Biotechnology 24:530-536 .

- Lee (1996). High cell density culture of E. coli. Trends in Biotechnology 14:98-105.

- Lund et al (2020). Understanding How Microorganisms Respond to Acid pH Is Central to Their Control and Successful Exploitation. Frontiers in Microbiology, Review.  
<https://doi.org/10.3389/fmicb.2020.556140>

- Protein Purification Handbook Amersham.

## **Software**

No software other than a proprietary software to control some bioreactors will be used in the lab sessions.

To process data and to write the lab reports the students can use a standard ofimatics package.