UAB
Universitat Autònoma de Barcelona

Integrated Laboratory 5

Code: 100924
ECTS Credits: 3

2025/2026

Degree	Type	Year
Biotechnology	OB	3

Contact

Name: Jaume Piñol Ribas

Email: jaume.pinyol@uab.cat

Teachers

Pau Ferrer Alegre

Teaching groups languages

You can view this information at the end of this document.

Prerequisites

It is recommended that students are taking or have already completed the theorical 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, theexpression 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.

Learning Outcomes

1. CM22 (Competence) Prioritise the instrumentation necessary for the different techniques for the separation and characterisation of biomolecules.
2. CM23 (Competence) Propose strategies for the purification of biomolecules from complex mixtures.
3. CM24 (Competence) Review the general safety standards of a biotechnology laboratory.
4. KM24 (Knowledge) Explain the theoretical foundation and appropriate techniques for the structural and functional characterization of proteins and nucleic acids.
5. SM20 (Skill) Use the basic techniques of manipulation, separation, detection and analysis of proteins and nucleic acids.
6. SM21 (Skill) Use prokaryotic and eukaryotic cell culture techniques and techniques for the manipulation of biological systems.
7. SM22 (Skill) Use analytical methodologies for the assay of biological activity of cellular components.

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 the of 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 de 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 de 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. Interpretingthe 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-PAG and enzyme activity of hDHFR purity and activity). Consistency of the results. Performance, puirification tables and purification factor.
- Session 14: Global analysis of the results. Evaluation of experimental data. Obtaining information to improve and redesign the production process

Activities and Methodology

Title	Hours	ECTS	Learning Outcomes
Type: Directed			
Lab classrooms	56	2.24	
Type: Supervised			
Tutorials	3	0.12	

Type: Autonomous		
Study	6	0.24
Writing lab reports	6	0.24

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.

Assessment

Continous Assessment Activities

Title	Weighting	Hours	ECTS	Learning Outcomes
Attitude and quality of work in the laboratory	10%	0	0	CM23, CM24, SM20
Written exam corresponding to sessions 1 to 7	30%	2	0.08	CM22, CM23, KM24, SM20, SM21,

				SM22
Written exam corresponding to sessions 8 to 14	25%	2	0.08	CM22, CM23, KM24, SM20, SM21, SM22
Written lab report corresponding to sessions 1 to 7	15%	0	0	CM22, CM23, KM24, SM20, SM21, SM22
Written lab report corresponding to sessions 8 to 14	20%	0	0	CM22, CM23, KM24, SM20, SM21, SM22

This subject cannot be evaluated by performing a single assessment. This 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) Puntuality, 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 does not 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 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).

The use of Artificial Intelligence (AI) technologies for the drafting of written reports is not allowed. Reports including AI-generated excerpts will be considered a lack of academic honesty and may result in a partial or total penalty in the grade of the activity, or greater penalties in cases of severity.

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 24530-536 .

- 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. Frntiers 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 experimental data and to write the lab reports the students can use a standard ofimatics package.

Groups and Languages

Please note that this information is provisional until 30 November 2025. You can check it through this link. To consult the language you will need to enter the CODE of the subject.

Name	Group	Language	Semester	Turn
(PLAB) Practical laboratories	431	Catalan/Spanish	first semester	afternoon
(PLAB) Practical laboratories	432	Catalan/Spanish	first semester	afternoon
(PLAB) Practical laboratories	433	Catalan/Spanish	first semester	afternoon
(PLAB) Practical laboratories	434	Catalan/Spanish	first semester	afternoon