Recombinant DNA Technology

Code: 100856
ECTS Credits: 3

2024/2025

Degree	Type	Year
2500252 Biochemistry	OB	3

Contact

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Teachers

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Teaching groups languages

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

Prerequisites

There are no official prerequisites. However, it is assumed that the student has acquired the basic knowledge of Molecular Biology explained in previous subjects of the degree of Biochemistry.

Objectives and Contextualisation

Recombinant DNA technology includes diferents methodologies developed from 1970-1980. These methodologies are now a basic tool in many biochemistry laboratories and have allowed in recent years a very important advance in the knowledge of the structure and function of biomolecules. In this subject the fundamentals of this technology will be presented. The general objective of the subject is to provide the knowledge that allows the student working with these methodologies during his professional future.

Specific objectives of the course:

- Know and apply the basic techniques of recombinant DNA: nucleic acid labeling, Southern and Northern blots, in situ hybridization, arrays, sequencing, restriction enzyme use, PCR reaction, CRISPR based technology.
- Describe the main cloning vectors in Escherichia coli, know their characteristics and know how to apply them in the different strategies for the cloning of DNA fragments.
- Understand strategies for the construction of libraries and their use for the study of genes and genomes.
- know the methodology for the expression of recombinant proteins and for the directed mutagenesis.

Competences

- Act with ethical responsibility and respect for fundamental rights and duties, diversity and democratic values.
- Apply the principal techniques used in biological systems: methods of separation and characterisation of biomolecules, cell cultures, DNA and recombinant protein techniques, immunological techniques, microscopy techniques, etc.
- Interpret experimental results and identify consistent and inconsistent elements.
- Introduce changes in the methods and processes of the field of knowledge to provide innovative responses to the needs and demands of society.
- Read specialised texts both in English and one's own language.
- 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.
- Use ICT for communication, information searching, data processing and calculations.

Learning Outcomes

1. Act with ethical responsibility and respect for fundamental rights and duties, diversity and democratic values.
2. Apply the basic techniques of recombinant DNA technology.
3. Design a basic protocol for obtaining mutants of a recombinant protein, expressing them and purifying them.
4. Design the cloning of a cDNA based on mRNA for the expression of recombinant protein.
5. Interpret experimental results and identify consistent and inconsistent elements.
6. Introduce changes in the methods and processes of the field of knowledge to provide innovative responses to the needs and demands of society.
7. Read specialised texts both in English and one's own language.
8. Take account of social, economic and environmental impacts when operating within one's own area of knowledge.
9. Take sex- or gender-based inequalities into consideration when operating within one's own area of knowledge.
10. Use ICT for communication, information searching, data processing and calculations.

Content

Unit 1. Introduction od recombinant DNA technology. Basic Tools of Recombinant DNA: restriction enzymes, polymerases, exonuclases, ligases, reverse transcriptase. cDNA synthesis, CRISPR system.

Unit 2. Hybridization techniques. Tm concept. DNA and RNA Labelling. Southern, Northern blot and their applications. Dot-Blot. in situ hybridization. Fish. Microarrays.

Unit 3. Polymer chain reaction (PCR) Introduction. Design and optimization of the reaction. RT-PCR. Quantitative PCR (real-time PCR). Applications.

Unit 4. Molecular Cloning. Blunt and cohesive extreme. Adaptors and Linkers. DNA Ligation. Bacterial transformation. Detection of recombinant clones. Characteristics of the cloning vectors: plasmids and bacteriophages. Some examples of plasmid. Specific vectors by alternative cloning systems: recombination integration systems, topoisomerase-based cloning systems.

Unit 5. Libraries of cDNA versus RT-PCR/RNA-seq. Strategies for the construction of libraries, concept of abundance and complexity of mRNA. Synthesis of cDNA. Main vectors used in the construction of cDNA libraries. Screening of cDNA libraries. RT-PCR / RNA-seq as an alternative to cDNA libraries.

Unit 6. Libraries for genomic sequencing. Construction and screening of genomic llibraries versus high throughput genomic sequence. Concept of Representativeness. Strategies for obtaining libraries for genomic sequencimg. Vectors used in genomic llibraries: Lambda, Cosmids, BACS. Screening of genomic DNA libraries. " Genomic Walking" and/or obtention of probe (reverse PCR). High throughput sequencing technologies.

Unit 7. Expression of recombinant proteins in E. coli. Kind of vectors used. Optimization of recombinant protein expression. Fusion proteins. In vitro translation systems. Site-directed mutagenesis vs Molecular evolution (Phage display).

Unit 8. Cloning in Yeast (S. cerevisae). Transformation. Typology of vectors. Expressions of recombinant proteinsin yeast. "Two-hybrid" method for detecting protein-protein interactions.

Activities and Methodology

Title	Hours	ECTS	Learning Outcomes
Type: Directed			
Magisterial class	16	0.64	2, 3, 4, 5, 7
Problem class	8	0.32	2, 10
Type: Supervised			
Tutorials	5	0.2	2, 3, 4, 5, 7, 10
Type: Autonomous			
Autonomous resolution of problems	15	0.6	2, 3, 4, 5, 10
Autonomous study (teoric class)	27	1.08	2, 3, 4, 7

The activities consist of theory and problem sessions.

Theory sessions

The teacher will explain the content of the syllabus with the support of audiovisual material that will be available to students in the Virtual Campus of the subject

Problem sessions

There will be 8 problem sessions per group. For these sessions, the theory group will be divided into two subgroups (A and B), the lists of which will be made public at the beginning of the course. Students will attend scheduled sessions for their group. At the beginning of the semester, a dossier will be delivered through the Virtual Campus with the problem statements, which will be solved by the teacher in a reasoned way and, if necessary, complementing part of the subjects explained in the theory classes.

Two of the problem sessions will be held in computer rooms in the afternoon. Each subgroup A and B will be divided into two (A1, A2, B1 and B2) and the sessions will take 2 hours.

Tutorials

Individual or small group tutorials will be held at the student's request. The objective of these tutorials will be to solve doubts, to orientate on the sources of information consulted and the preparation of the seminars. In case

the number of requests is extremely high, especially before exams, a classroom tutorial could be held before exams, to solve doubts or to review basic concepts, which will be announced in due time through the Virtual Campus. These sessions will not be expository nor will the official syllabus be advanced, but will be sessions of debate and discussion.

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
Individual written test of problems	25 %	1.5	0.06	1, 2, 3, 4, 5, 6, 7, 8, 9, 10
Theory module evaluation: multiple choice questions	40%	1	0.04	1, 2, 3, 4, 5, 6, 7, 8, 9, 10
Written theory test of short questions	35%	1.5	0.06	1, 2, 3, 4, 5, 6, 7, 8, 9, 10

In order to evaluate the level of assimilation of knowledge, as well as the capacity to relate concepts, critical reasoning and other transversal competences, a series of evaluative activities of different typologies will be carried out.
These evaluative activities will be identical for both the continuous evaluation system and the single evaluation system.

Continuous evaluation

Evaluation of the theory module (75%):

It will consist of two evaluative activities of different typologies.

1) Individual evaluation by means of pot-type questions (multiple-choice).

2) Individual evaluation by means of short open-ended questions relating several sections of the program, on the use of knowledge to interpret experimental results or to propose the most appropriate techniques to reach the objectives set out in the question.

There will be no minimum grade to make the average between the two types of evaluative activities and to obtain the final grade of the theory module.
The overall weight of the theory module will be 75% of the final grade of the course, and the test will be given on the date scheduled in the calendar.
This test may be recovered on the day set for the recovery of the subject

Evaluation of problems module (25%).

An individual evaluation will be carried out by means of a written test. It will consist of the resolution of 2 problems posed by the teacher of the same type as those worked during the formative activity of problems.
The weight of this test will be 25% of the final grade of the course and will be done on the date scheduled in the calendar.
This test may be recovered on the day set for the recovery ofthe subject.

Single Evaluation

Theory module evaluation (75%):

The single evaluation test of this module will be the same as in the continuous evaluation and will be done coinciding with the same date set in calendar as in the continuous evaluation.
The same recovery system will be applied as for the continuous evaluation.

Problem module evaluation (25%).

The written test of single evaluation of this module will be the same and will have the same weight as in the continuous evaluation (25%).
It will coincide with the same date fixed in the calendar as in the continuous evaluation, and the same recovery system will be applied as in the continuous evaluation.

General considerations for the two continuous and single evaluation systems

- The evaluation of the modules of Theory and Problems are inseparable, and in order to pass the course, the student must participate and be evaluated in both modules.
- The written tests of theory and problems will be taken together on the dates scheduled and already fixed in the calendar.
- The grade is obtained by the weighted average of each of the modules. In order to pass the course, it is necessary to obtain a final grade equal or higher than 5.
- Students who do not pass the two tests together with a grade equal to or higher than 5 will be able to recover them on the date scheduled for the recovery exam at the end of the semester.
- To participate in the recovery, the student must have been previously evaluated in a set of activities, the weight of which is equivalent to a minimum of two thirds of the total grade of the subjectormodule.
- The student will obtain the grade of "Not Evaluable" when the evaluation activities carried out have a weight of less than 67% in the final grade.
- Students who wish to improve their grade may take the grade improvement exam at the end of the semester, which will take place on the date scheduled for the make-up exam. Students who take the make-up exam will forfeit the grade obtained in the written tests taken during the course.
- Students who cannot attend an individual evaluation test for justified cause and provide the corresponding official documentation to the grade coordination, will have the right to take the test in question on another day. The coordination of the degree will ensure the concretion of this with the professor of the affected subject.
- Any aspect not covered in this guide will follow the evaluation regulations of the Faculty of Biosciences.

Bibliography

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2) MOLECULAR BIOTECHNOLOGY, PRINCIPLES AND APPLICATIONS OF RECOMBINANT DNA Harris, Bernadette;Harris, BernadetteeBook eBook | 2018.
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3) Nicholl, Desmond S. T. An Introduction to genetic engineering 2008. eBook 2n edition (2002).
https://bibcercador.uab.cat/permalink/34CSUC_UAB/15r2rl8/cdi_proquest_miscellaneous_18821168

4) S. B. Primrose and R. M. Twyman Principles of gene manipulation and genomics /, SEVENTH EDITION, eBook | 2006.
https://bibcercador.uab.cat/permalink/34CSUC_UAB/15r2rl8/cdi_askewsholts_vlebooks_9781444309096

5) H. Freeman. Recombinant DNA : genes and genomes - a short course, 2007

6) J. Perera, Julián. Ingeniería genética 2002

Software

Microsoft Word, PowerPoint, Excel

Primer Design: Serial Cloner 2.6, NetPrimer, Primer3Plus, Primer-BLAST, PrimerX.

Language list

Name	Group	Language	Semester	Turn
(PAUL) Classroom practices	331	Catalan/Spanish	first semester	morning-mixed
(PAUL) Classroom practices	332	Catalan/Spanish	first semester	morning-mixed
(PLAB) Practical laboratories	331	Catalan/Spanish	first semester	afternoon
(PLAB) Practical laboratories	332	Catalan/Spanish	first semester	afternoon
(PLAB) Practical laboratories	333	Catalan/Spanish	first semester	afternoon
(PLAB) Practical laboratories	334	Catalan/Spanish	first semester	afternoon
(TE) Theory	33	Catalan/Spanish	first semester	morning-mixed