Universitat Autònoma de Barcelona

2022/2023

Recombinant DNA Technology

Code: 100856
ECTS Credits: 3

Degree	Type	Year	Semester
2500252 Biochemistry	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: No
Some groups entirely in Spanish: No

Teachers

Josep Antoni Perez Pons

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.

Methodology

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 the computer room.

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			
Magisterial class	16	0.64	2, 4, 3, 5, 7
Problem class	8	0.32	10, 2
Type: Autonomous			
Autonomous resolution of problems	15	0.6	10, 2, 4, 3, 5
Autonomous study (teoric class)	32	1.28	2, 4, 3, 7

Assessment

Theory module evaluation (70%): It will consist of two evaluation activities:

1) Individual evaluation: Multiple choice questions (40%)

2) Individual evaluation: Written exam based on short questions (30%)

Problem module evaluation (30%).

Individual evaluation: Resolution of 2 problems.

General Connsideracions

The grade is obtained by the weighted average of each of the modules when the grade is equal to or greater than 4.

To pass the subject it is necessary to obtain a final grade equal to or greater than 5

The students will obtain the grade of "Not Evaluable" when the evaluation activities carried out have a weight lower than 67% in the final grade

Any aspect that is not contemplated in this guide will follow the evaluation regulations of the Faculty of Biosciences.

Assessment Activities

Title	Weighting	Hours	ECTS	Learning Outcomes
Individual written test of problems	30 %	1.5	0.06	1, 9, 8, 10, 2, 4, 3, 5, 6, 7
Theory module evaluation: multiple choice questions	35%	1	0.04	1, 9, 8, 10, 2, 4, 3, 5, 6, 7
Written theory test of short questions	35%	1.5	0.06	1, 9, 8, 10, 2, 4, 3, 5, 6, 7

Bibliography

1) Gene Cloning and DNA Analysis : An Introduction T. A. Brown;T. A. Brown eBook | 2016

https://login.are.uab.cat/login?url=http://search.ebscohost.com/login.aspx?direct=true&db=edsebk&AN=1081525

2)MOLECULAR BIOTECHNOLOGY, PRINCIPLES AND APPLICATIONS OF RECOMBINANT DNA Harris, Bernadette;Harris, BernadetteeBook | 2018

https://login.are.uab.cat/login?url=http://search.ebscohost.com/login.aspx?direct=true&db=edsebk&AN=2267894

3)Nicholl, Desmond S. T. An Introduction to genetic engineering eBook | 2008

https://login.are.uab.cat/login?url=http://search.ebscohost.com/login.aspx?direct=true&db=edsebk&AN=304648&

4) S. B. Primrose and R. M. Twyman Principles of gene manipulation and genomics /, SEVENTH EDITION, eBook | 2006

https://login.are.uab.cat/login?url=http://search.ebscohost.com/login.aspx?direct=true&db=edsebk&AN=274676&

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.