Universitat Autònoma de Barcelona

2020/2021

Recombinant DNA Technology

Code: 100856
ECTS Credits: 3

Degree	Type	Year	Semester
2500252 Biochemistry	OB	3	1

The proposed teaching and assessment methodology that appear in the guide may be subject to changes as a result of the restrictions to face-to-face class attendance imposed by the health authorities.

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

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

- 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.
- Collaborate with other work colleagues.
- Interpret experimental results and identify consistent and inconsistent elements.
- Make an oral, written and visual presentation of ones work to a professional or non-professional audience in English and understand the language and proposals of other specialists.

- Read specialised texts both in English and ones own language.
- Use ICT for communication, information searching, data processing and calculations.

Learning Outcomes

1. Apply the basic techniques of recombinant DNA technology.
2. Collaborate with other work colleagues.
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. Make an oral, written and visual presentation of ones work to a professional or non-professional audience in English and understand the language and proposals of other specialists.
7. Read specialised texts both in English and ones own language.
8. 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.

Unless the requirements enforced by the health authorities demand a prioritization or reduction of these contents.

Methodology

The activities consist of theory and problem classes.

Magisterial class

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 classes

There will be 8 sessions of problems per group, in the date announced in the calendar. For these sessions, the theory group will be divided into two subgroups. Sone of the sessions of the problems will be done in the computer room.

The proposed teaching methodology may experience some modifications depending on the restrictions to face-to-face activities enforced by health authorities.

Activities

Title	Hours	ECTS	Learning Outcomes
Type: Directed			
Magisterial class	16	0.64	1, 4, 3, 5, 7
Problem class	8	0.32	8, 1, 2, 6
Type: Autonomous			
Autonomous resolution of problems	15	0.6	8, 1, 4, 3, 5
Autonomous study	32	1.28	1, 4, 3, 7

Assessment

Theory module evaluation (65%): It will consist of two evaluation activities:

1) Individual evaluation: multiple choice questions (35%)

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

Problem module evaluation (35%). it will consist of two evaluation activities:

1. Resolution of the problems worked as autonomus way and exposed in class (15% of the final grade).

2. Individual evaluation: It will consist of the resolution of 1-2 problems (20% of the total).

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.

Student's assessment may experience some modifications depending on the restrictions to face-to-face activities enforced by health authorities.

Assessment Activities

Title	Weighting	Hours	ECTS	Learning Outcomes
Problem module evaluation : Resolution of the problems in class	15 %	1	0.04	8, 1, 4, 3, 5, 7
Problem module evaluation: written exam (resolution of 1-2 problems)	20%	1	0.04	8, 1, 4, 3, 5, 7
Theory module evaluation: multiple choice questions	35%	1	0.04	1, 4, 3, 5, 7
Theory module evaluation: written exam based on short questions	30%	1	0.04	8, 1, 2, 4, 3, 5, 7, 6

Bibliography

The bibliography and the web links are updated in the Teaching Material section of the Virtual Campus.