Universitat Autònoma de Barcelona

2021/2022

Recombinant DNA Technology

Code: 100934
ECTS Credits: 3

Degree	Type	Year	Semester
2500253 Biotechnology	OB	2	2

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: Yes
Some groups entirely in Spanish: No

Prerequisites

No special requirements are requested.

Objectives and Contextualisation

This subject is aimed to introduce the student to the wide range of methodologies that are commonly known as Recombinant DNA Technology. These methodologies, most of them developed at the end of the last century, are one of the pillars of modern biotechnology. The general objective is to provide a solid basis allowing the student to apply these methodologies when designing biotechnological processes. In addition, it is also provided the scientific background to follow-up more specialized topics in the last courses of the degree of Biotechnology. The practical aspects of this subject are dealt in the Integrated Laboratory 5.

- Known and know how to apply the basic techniques of recombinant DNA and nucleic acid engineering: enzymatic tools (restrictases, polymerases, kinases, phosphatases, ligases, topoisomerases, site-specific recombinases and non-specific nucleases), different types of PCR reactions, probe labeling, Southern and Northern blot.

- Describe the main cloning vectors, know their main characteristics and know how to use them in the different strategies for the cloning of DNA fragments.

- Understand the strategies for the construction of libraries and their use for the study of genes and genomes.

- Know the basics and main applications of the new technologies for massive sequencing of nucleic acids (Next Generation Sequencing).

- Describe the main applications of recombinant DNA to obtain mutations: site directed mutagenesis, random mutagenesis and methods for directed molecular evolution.

- Know the methodology for the expression of recombinant proteins.

Competences

- 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.
- Read specialised texts both in English and ones own language.
- Think in an integrated manner and approach problems from different perspectives.
- Work individually and in teams

Learning Outcomes

1. Describe and apply the different methods for obtaining mutants of a recombinant protein and purifying these.
2. Describe the strategies used for modifying the genome of different organisms.
3. Design and execute cDNA cloning for the study of gene expression and for recombinant protein expression.
4. Design strategies for genome sequencing.
5. Master the basic methods of recombinant DNA technology.
6. Read specialised texts both in English and ones own language.
7. Think in an integrated manner and approach problems from different perspectives.
8. Work individually and in teams

Content

1. Basic techniques in recombinant DNA technology. What can we do with the recombinant DNA technology? Main enzymes used in recombinant DNA technology. DNA restriction. Adaptors and linker-adaptors. DNA denaturation and molecular hybridization. The PCR reaction and primer designing. Sanger reaction, Southern Blot, Northern Blot and its applications.

2. Cloning in ***Escherichia coli***. Plasmids and phages as cloning vectors in *E. coli*. Main transformation methods. Phagemids and main host strains. Integration by recombination. Cloning of PCR products.

3. Cloning of cDNAs. cDNA synthesis. Strategies to create representative cDNA libraries. Main vectors used to build cDNA libraries. In vivo excision system in lambda phages. Screening cDNA libraries. Reverse Transcription PCR (RT-PCR) and Rapid Amplification of cDNA ends (RACE). Historical note about nucleic acid arrays. Next generation sequencing technologies (NGS). RNASeq strategies to discover expressed genes and characterize gene expression.

4. Genomic DNA libraries. General concept. Rules to obtain representative libraries. Strategies to build genomic DNA libraries. Cosmid and fosmids, BACS, PACS and YACS. Screening of genomic libraries. Arrangement of contigs. Application of NGS to "de novo" sequencing and resequencing of genomes.

5. ***In vitro*** mutagenesis. Concept and uses. Silent mutations. Site directed mutagenesis (SDM). Metods for SDM (cassette, primer extension and PCR). Random mutagenesis. Molecular directed evolution (DNA shuffling and related technologies).

6. Expression of recombinant proteins. Factors affecting the expression of cloned genes in *E. coli*. Main expression vectors. Optimization of recombinant protein expression. Synthetic genes. Fusion proteins. Phage display. In vitro translation systems.

Methodology

The training activities consist of theory classes and practical sessions for problem and case solving. Each of them has its own specific methodologies.

Theory classes
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 (VC) of the subject. These lectures will be the most important part of the theory section. It is recommended that students have the material published on the VC in printed or digital form in order to be able to follow the teacher more comfortably. Under the guidance of the teacher, the study of some parts of the syllabus will have to be deepened by the students, by means of autonomous learning. In order to facilitate this task, teaching materials will be provided in the form of textbooks, scientific literature, web pages, etc.

Practical sessions for problem and case solving
The class group will be divided into two subgroups (A and B). It is expected that 8 sessions will be devoted to the resolution of practical cases and experimental problems related to the contents of the theory program. Students will attend the sessions programmed for their group. At the beginning of the semester a dossier of questions, problems and cases will be available at the VC. These practical problems and cases will be solved throughout the sessions. In these sessions the problem professor will present the experimental and calculation principles necessary to work on the problems, explaining the guidelines for their resolution and reinforcing at the same time the knowledge of different parts of the subject from theory classes. It is expected the active participation of the students.

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			
Practical sessions for problem solving	8	0.32	1, 3, 5, 7, 8
Theory classes	17	0.68	1, 2, 3, 5, 6, 7, 8
Type: Autonomous			
Study and individual work to prepare problems and cases	44	1.76	1, 2, 4, 3, 5, 6, 7, 8

Assessment

The subject will be evaluated continuously during the course. 10% of the overall grade will be calculated based on the amount and quality of the contributions made by each student in the VC forum of the subject. There will also be two exams. The first one will have a 50% weight in the overall grade and it will be a quiz that may also include short questions and it will essentially evaluate the contents of the theory classes. The second exam, with a weight of 40% in the overall grade, will be a exam about the Practical sessions for problem solving and the student will have to solve different exercises and / or problems similar to those done during the practical sessions.
In the event that the student does not pass the subject, he might attend a second examination. However, to participate in the second examination, the students must have been previously evaluated in a set of activities whose weight equals to a minimum of two thirds of the total grade of the subject. The second examination has the same overall distribution of tests tha the first eaxmination. All students are welcome to this second examination and note does not imply the resignation of the previous marks. The mark obtained in the participation in the VC forum cannot be reevaluated.

The overall grade of the subject will be calculated as follows:

- 50% of the theory exam (or the recovery of the same if applicable).

- 40% of the classroom practices exam (or recovery of the same if applicable).

- 10% of the participation in the forum of the subject.

No minimum marks will be required to be able to pass each of the assessment activities. In order to pass the subject, the student must achieve at least a global score of 5.0. The students will obtain a "No Avaluable" mark when the evaluation activities carried out have a weighting less than 67% in the final grade.

Assessment Activities

Title	Weighting	Hours	ECTS	Learning Outcomes
Exam of problem and case solving	40%	2.5	0.1	1, 4, 5, 6, 7, 8
Participation in the VC forum of the subject	10%	2.5	0.1	1, 2, 4, 3, 5, 6, 7, 8
Theory exam	50%	1	0.04	1, 2, 4, 3, 5, 6, 7, 8

Bibliography

Textbooks:

- Molecular Biotechnology. Principles and Applications of Recombinant DNA. 5th Ed. B.R. Glick & C.L. Patten. ASM Press, 2018.

- Gene Cloning & DNA Analysis. An Introduction. 8th Ed. T.A. Brown. Wiley Blackwell, 2020.

In Campus Virtual is available a reference list about specific items of this subject

Software

No specific software is used in this subject