Recombinant DNA Technology

Code: 100934
ECTS Credits: 3

2024/2025

Degree	Type	Year
2500253 Biotechnology	OB	2

Contact

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Teaching groups languages

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

Prerequisites

No special requirements are requested. However it is highly recommended that students have been previously enrolled in several core subjects such as Biochemistry (1st year) and Molecular Biology (2nd year, 2nd semester).

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 foundations of the 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.

- Know and know how to apply the basic techniques of recombinant DNA and nucleic acid engineering: enzymatic tools (restriction enzymes, polymerases, kinases, phosphatases, ligases, topoisomerases, site-specific recombinases and non-specific nucleases), different types of PCR reactions, probe labeling and 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 to modify DNA sequences: site directed mutagenesis, random mutagenesis and methods for directed molecular evolution.

- Know the main methods to obtain synthetic genes and for the expression of recombinant proteins.

Learning Outcomes

1. CM13 (Competence) Interpret the basic methods of recombinant DNA technology.
2. CM15 (Competence) Work collaboratively in teams to solve problems in the field of biochemistry.
3. KM15 (Knowledge) Provide a proper description of the differential regulation of gene expression in prokaryotes and eukaryotes.
4. SM14 (Skill) Correctly interpret data and observations in the field of biochemistry.
5. SM14 (Skill) Correctly interpret data and observations from the field of biochemistry.

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.

Activities and Methodology

Title	Hours	ECTS	Learning Outcomes
Type: Directed			
Practical sessions for problem solving	8	0.32	
Theory classes	17	0.68	
Type: Autonomous			

Study and individual work to prepare problems and cases	44	1.76

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.

Assessment

Continous Assessment Activities

Title	Weighting	Hours	ECTS	Learning Outcomes
Exam of problem and case solving	40%	2.5	0.1	CM13, KM15, SM14
Participation in the VC forum of the subject	10%	2.5	0.1	CM15
Theory exam	50%	1	0.04	CM15

Continuous assessment

Two exams will be held. The first exam will have a weight of 50% in the overall qualification and will be a quiz that may also include short questions and will evaluate the contents of the theory classes. The second test, with a weight of 40% in the overall qualification, will be a classroom practice exam and the student will have to solve different exercises and/or problems similar to those carried out during practice classes. The global qualification will be completed by the contributions of the students in the subject's forum continuously throughout the course. This part will have a weight of 10% of the overall grade and the grade will be calculated based on the quantity and quality of the contributions made by the students.

In the event that the student does not pass the subject, he/she might attend a second round of exams. However, to participate in this second round, 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 round

has the same overall distribution of tests that the first round. All students are welcome to this second exam round. Participation in this round does not imply the resignation of the previous marks. The mark obtained in the participation in the VC forum cannot be reevaluated. However, students failing the subject might chose to conserve the mark obtanied in the VC forum for a maximum of one academic year.

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

- 50% of the theory exam (in the first or the second round, if applicable). A minimum score of 3.5 is required.

- 40% of the problem solving exam (in the first or the second round, if applicable). A minimum score of 3.5 is required.

- 10% of the participation in the forum of the subject. No miminum score is required.

In order to pass the subject, the student must achieve at least a global score of 5.0 and scores equal or higher than 3,5 in the exams. The students will obtain a "No Avaluable" mark when the evaluation activities carried out have a weighting less than 67% in the final grade.

Single assessment

Students adhering to the single assessment mode will have to sit the exams described above for the continuous assessment. In the case of performing a single assessment, participation of students in the subject's forum (10% of the final grade) will be replaced by a written revision work on a current topic of the subject. The topic of this written work will be arranged during the first two weeks after starting the semester. The teacher will provide the necessary bibliography and the work must be submitted in printed form on the day of the exam. Failure to agree on the topic of the written work will result in a grade of 0 in this section.

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

Language list

Name	Group	Language	Semester	Turn
(PAUL) Classroom practices	421	Catalan	second semester	afternoon
(PAUL) Classroom practices	422	Catalan	second semester	afternoon
(TE) Theory	42	Catalan	second semester	afternoon