Universitat Autònoma de Barcelona

2021/2022

Recombinant DNA: Basics and Advanced Applications

Code: 42895
ECTS Credits: 9

Degree	Type	Year	Semester
4313794 Biochemistry, Molecular Biology and Biomedicine	OT	0	A

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: spanish (spa)

Other comments on languages

English is the language of most of the provided didactic material and of some sessions of the module

Teachers

Joaquín Ariño Carmona
Josep Antoni Biosca Vaqué
Inmaculada Ponte Marull
Antonio Casamayor Gracia
Jaume Piñol Ribas
Nerea Roher Armentia
Irantzu Pallarés Goitiz
Alicia Roque Córdova Roque Cordova

External teachers

Jordi Moreno Romero
Marcus Buschbeck

Prerequisites

For graduates in Biochemistry, Biology, Biomedical Sciences, Genetics, Microbiology, Chemistry, Computer Science, Physics, Veterinary Medicine and Pharmacy

In any case, it is recommended to learn the basic techniques of recombinant DNA.

Objectives and Contextualisation

The main goal of the course is to provide an advanced and rigorous training about a diversity of recombinant DNA techniques, both basic and advanced. So, at the end of the module the student will have achieved a solid knowledge of different techniques involving the manipulation of recombinant DNA currently used in research laboratories as well as profits and limitations.

After completing this module, students will be able to:

1. Understand the methodological procedures and identify current instrumental tools based on recombinant DNA technology to address key issues in many research areas, such as the structure of DNA, the structure and function of chromatin, the evaluation of the expression and regulation, translation, and the subcellular localization of proteins, etc…

2. Design and conduct experiments using the most appropriate for each specific objective experimental recombinant DNA techniques.

3. Analyze and properly interpret and critically evaluate both, own and published in the scientific literature, experimental data.

4. Defining and understanding the specific techniques for specific organisms used as experimental models in research laboratories as well as profits and limitations.

Competences

- Analyse and correctly interpret the molecular mechanisms operating in living beings and identify their applications.
- Analyse research results to obtain new biotechnological or biomedical products to be transferred to society.
- Apply techniques for modifying living beings or parts of these in order to improve pharmaceutical and biotechnological processes and products or develop new products.
- Communicate and justify conclusions clearly and unambiguously to both specialist and non-specialist audiences.
- Continue the learning process, to a large extent autonomously.
- Develop critical reasoning within the subject area and in relation to the scientific or business context.
- Identify and propose scientific solutions to problems in molecular-level biological research and show understanding of the biochemical complexity of living beings.
- Integrate contents in biochemistry, molecular biology, biotechnology and biomedicine from a molecular perspective.
- Solve problems in new or little-known situations within broader (or multidisciplinary) contexts related to the field of study.
- Use and manage bibliography and IT resources related to biochemistry, molecular biology or biomedicine.
- Use scientific terminology to account for research results and present these orally and in writing.
- Work individually and in teams in a multidisciplinary context.

Learning Outcomes

1. Analyse research results to obtain new biotechnological or biomedical products to be transferred to society.
2. Analyse, correctly interpret and critically assess both one's own experimental data and those published in the scientific literature.
3. Communicate and justify conclusions clearly and unambiguously to both specialist and non-specialist audiences.
4. Continue the learning process, to a large extent autonomously.
5. Decide on the most appropriate organism to use for each specific need.
6. Design and conduct experiments using the most appropriate experimental techniques of recombinant DNA for each particular objective.
7. Develop critical reasoning within the subject area and in relation to the scientific or business context.

8. Distinguish the bases of the most commonly used standard techniques in molecular biology.
9. Make the necessary changes to improve performance.
10. Solve problems in new or little-known situations within broader (or multidisciplinary) contexts related to the field of study.
11. Understand methodological procedures and the advantages and limitations of currently-used instruments for conducting research in this field (chromatin structure, gene expression and its regulation, mRNA processing, etc.).
12. Use and manage bibliography and IT resources related to biochemistry, molecular biology or biomedicine.
13. Use scientific terminology to account for research results and present these orally and in writing.
14. Work individually and in teams in a multidisciplinary context.

Content

The content of this module is as follows*:

1) Introduction to basic molecular biology techniques.

1.1. Principles of gene cloning and DNA analysis.

- Amplification, labeling and detection of nucleic acids.
- Type of vectors, DNA molecular cloning strategies and gene libraries of DNA.
- DNA-directed mutagenesis.

1.2. Applications of gene cloning and DNA analysis for the study of gene expression.

- Techniques for studying gene expression based on DNA microarrays and massive sequencing of DNA (RT-PCR, Run On, microarrays and DNA footprinting, promoter analysis using reporter genes, mRNAs massive sequencing December, ChIP-Seq, GRO-Seq, etc.)..

2) Characteristics of commonly used model organisms.

3) Techniques for the study of epigenetic mechanisms that regulate chromatin structure and its role in the replication, transcription and repair of eukaryotic DNA.

- Determination of heterochromatin and euchromatin regions (microscopic / sensitivity to nucleases / density gradients / chromatin precipitation curves, etc.).

- Modifications of histones (histone code). ChIP, ChIP-chip, ChIP-seq and others.

- DNA methylation. Identification of methylation in a sequence. Characterization of the degree of methylation at CpG islands. Building the methyloma.

- Methods for characterizing high and low resolution nucleosome positioning and identification of sites of hypersensitivity to nucleases (End labeling, in vivo footprinting, LM-PCR, etc.).

- Methodology for the study of chromatin remodeling complexes.

- Techniques for studying the 3D organization of the genome (Hi-C).

- Applications of epigenetics in the analysis of genomic imprinting in plants.

4) Modification of genomes and gene silencing (RNA antisense techniques, KO, ribozymes, GM, using adjustable promoters, etc.).

5) Protein Expression. Characteristics of various protein expression systems (types of vectors, promoters, organizations,etc.). Study of the intracellular localization of proteins.

6) Detection of protein-protein interaction (double hybrid protein chips, FRET, etc.) and Interactomics.

7) Applications of recombinant DNA technology in industry and medicine (diagnosis, antibody engineering, metabolic, etc.).

8) Presentation and defense of a bibliographic work.

9) Case reports.

10) Practical sessions: 24h of laboratory work divided in 8 sessions of variable length (2-4h/session). *Yeast two hybrid*, PCR, RNA-seq analysis, chromatin immunoprecipitation, q-PCR, sequence databases and analysis tools.

11) Experts seminaries.

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

Methodology

Part of the teaching will be in the classroom and will comprise lectures, laboratory work and assistance in the defense of bibliographical works as detailed below. It is excluded the attendance at scheduled seminars, part of Module 2 Advanced seminars in Biochemistry and Molecular Biology.

Face class / Directed activities (60 h)

- Lectures and seminars 32 h

- Laboratory sessions 24 h

- Presentation and defense of bibliographical 4 h

Another part will be self-study by students, including the execution of tests and exercises throughout the course.

Autonomous

- Student Self Study: 67.5 h

- Theoretical and practical tests throughout the course (resolution of cases and problems): 24 h

The preparation by the students of a bibliographic work is the main activity supervised.

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

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			
Classroom lectures	32	1.28	2, 5, 7, 8, 11, 9, 4
Laboratory sessions	24	0.96	2, 6, 8, 11, 10, 3, 4, 14, 12
Supervised	4	0.16	1, 2, 7, 8, 11, 10, 4, 14, 12, 13
Type: Supervised			
Preparation of a bibliographic work	47	1.88	1, 2, 7, 8, 11, 10, 4, 14, 12, 13
Type: Autonomous			
Autonomous learning	67.5	2.7	1, 2, 5, 7, 6, 8, 11, 10, 4, 14, 12
Theoretical and practical tests throughout the course (resolution of cases and problems)	24	0.96	2, 5, 7, 6, 8, 11, 9, 10, 3, 4, 14, 12

Assessment

In order to pass the module the grade obtained by the weighted average of the different activities must be at least of 5 points.

A retake exam will be available for those students who didn't get an average mark greater than 5 points. To be eligible for the retake process, the student should have been previously evaluated in a set of activities equaling at least two thirds of the final score of the course or module. Thus, the student will be graded as "No Avaluable" if the weighting of all conducted evaluation activities is less than 67% of the final score.

Important: If plagiarism is detected in any of the works submitted, the student will fail the whole module.

*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
Laboratory sessions	30%	2	0.08	1, 2, 5, 6, 8, 11, 9, 10, 3, 4, 14
Oral presentation of the bibliographic work	40%	0.5	0.02	1, 2, 5, 7, 6, 8, 11, 9, 10, 3, 4, 14, 12, 13
Resolution of exercises presented by teachers, throughout the course	30 %	24	0.96	1, 2, 5, 7, 6, 8, 11, 10, 14, 12, 13

Bibliography

* Molecular Cloning: A Laboratory Manual.

John J. Sambrook, David David William Russell.

Cold Spring Harbor Laboratory Press; 4th edition. 2012.

* Current Protocols in Molecular Biology

Ausubel et al.

J. Willey, 2012. https://doi.org/10.1002/0471142727.mbprefs98

* Gene Cloning and DNA Analysis: An Introduction (6th edition).

T.A. Brown.

Wiley-Blackwell; 6th edition, 2013.

* Lewin's GENES XII.

Jones & Bartlett Learning. 2017.

* Molecular Biotechnology: Principles and Applications of Recombinant DNA.

Bernard R. Glick, Jack J. Pasternak, Cheryl L. Patten.

ASM Press; 5th edition. 2017.

* Next-Generation DNA Sequencing Informatics.

Stuart M. Brown

Cold Spring Harbor Laboratory Press, 2013.

* Diverses revisions en revistes com: Current Opinion in Structural Biology, Trends in Biochemical Sciences, Trends in Biotechnology, Nature Biotechnology, Nature Methods, etc.

Software

Databases and analysis tools used in this subject are listed below:

Databases:

- NCBI Gene https://www.ncbi.nlm.nih.gov/gene/

- SGD (https://www.yeastgenome.org/)

- Expasy (https://www.expasy.org/)

Analysis tools:

- BLAST (https://blast.ncbi.nlm.nih.gov/)

- Snapgene (https://www.snapgene.com/),

- GraphPad

- Bowtie2 v2.4.4 (http://bowtie-bio.sourceforge.net/bowtie2/index.shtml)

    - bowtie2-align-l.exe
    - bowtie2-align-s.exe

- bowtie2-build-l.exe
- bowtie2-build-s.exe
- bowtie2-inspect-l.exe
- bowtie2-inspect-s.exe
- Running scripts in Python and Perl

- BWAmem V.0.7.17 (https://sourceforge.net/projects/bio-bwa/files/

- Cutadapt V3.4, running in Python 3.0. (https://cutadapt.readthedocs.io/en/stable/)

- featureCounts v2.0.2 (https://sourceforge.net/projects/subread/files/subread-2.0.2/)

- Glimmer3 V3.02 (http://ccb.jhu.edu/software/glimmer/index.shtml)

- Java

- Varna393.jar (http://varna.lri.fr/index.php?lang=en&page=downloads&css=varna)
- gview.jar V1.7 (https://github.com/phac-nml/gview-wiki/wiki/Downloads)
- VarScan.v2.3.4.jar (https://sourceforge.net/projects/varscan/files/)

- mEMBOSS suite V6.5 (ftp://emboss.open-bio.org/pub/EMBOSS/)

- vectorstrip
- Getorf

- NCBI blast suite V2.11 (https://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/LATEST/)

- blast_formatter.exe
- blastn.exe
- blastp.exe
- blastx.exe
- makeblastdb.exe

- Primer3 V2.4.0 (https://sourceforge.net/projects/primer3/files/primer3/2.4.0/)

- R-4.0.0

- ContigStats.R (https://gist.github.com/jlhg/4642041)
- FastqQuality.R (http://www.sthda.com/english/wiki/fastqcr-an-r-package-facilitating-quality-controls-of-sequencing-data-fo
- scrDESeq2.R (https://bioconductor.org/packages/release/bioc/html/DESeq2.html)
- tradis_essentiality.R (https://github.com/sanger-pathogens/Bio-Tradis/blob/master/bin/tradis_essentiality.R)

- Samtools V1.12 (http://www.htslib.org/download/)

- SPAdes 3.15.2 (https://cab.spbu.ru/software/spades/)

- trf409.exe (https://tandem.bu.edu/trf/trf.download.html)

- Velvet V1.2.10 (https://www.ebi.ac.uk/~zerbino/velvet/)

- velvetg.exe
- velveth.exe