COURSE DATA

DATA SUBJECT

Code: 33178
Name: Methods in molecular biology and genetic engineering
Cycle: Undergraduate Studies
ECTS Credits: 4.5
Academic year: 2026-27

STUDY (S)

Degree	Center	Acad. year	Period
1111 - Grado en Biotecnología	Facultat de Ciències Biològiques	3	Second quarter

SUBJECT-MATTER

Degree	Subject-matter	Character
1111 - Grado en Biotecnología	Cellular and molecular methodology	COMPULSORY

COORDINATION

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SUMMARY

The aim of this course is that students acquire the basic conceptual and methodological knowledge concerning:

(1) The basic tools for analysis of nucleic acids.

(2) Development of the basic tools for cloning.

(3) Characterization and modification of DNA sequences and DNA manipulation on a large scale.

(4) Genomic sequencing. Combination of massive automated sequencing methods and bioinformatics techniques to address the sequencing of entire genomes.

(5) Other widely used techniques in molecular biology and gene fusions, methods for analyzing interaction between proteins and between proteins and nucleic acids, etc.

PREVIOUS KNOWLEDGE

RELATIONSHIP TO OTHER SUBJECTS OF THE SAME DEGREE

There are no specified enrollment restrictions with other subjects of the curriculum.

OTHER REQUIREMENTS

Students should have completed in the previous semester (or in previous years) "Molecular Biology" in order to properly understand the contents of this subject.

COMPETENCES / LEARNING OUTCOMES

1102 -

Be able to use recombinant DNA techniques and design protocols.

Properly handle the equipment and material of a biochemistry and molecular biology laboratory.

Saber realizar análisis de expresión génica.

1111 - Grado en Biotecnología

Actuar con autonomía en el aprendizaje, tomando decisiones fundamentadas en diferentes contextos, emitiendo juicios en base a la experimentación y el análisis y transfiriendo el conocimiento a nuevas situaciones

Apply analytical, synthetic and critical thinking skills in the application of the scientific method.

Colaborar eficazmente en equipos de trabajo, asumiendo responsabilidades y funciones de liderazgo y contribuyendo a la mejora y desarrollo colectivo

Conocer las bases químicas y moleculares del funcionamiento celular

Conocer las técnicas básicas que se utilizan para los estudios de expresión génica y para la manipulación del material genético.

Conocer y comprender, desde el propio ámbito de la titulación, las desigualdades por razón de sexo y género en la sociedad; integrar las diferentes necesidades y preferencias por razón de sexo y de género en el diseño de soluciones y resolución de problemas

Contribuir en el diseño, desarrollo y ejecución de soluciones que den respuesta a demandas sociales, teniendo en cuenta como referente los Objetivos de Desarrollo Sostenible

Demostrar razonamiento crítico y autocrítico en el ámbito de la titulación, considerando aspectos tales como la ética profesional, los valores morales y las implicaciones sociales de las diferentes actividades realizadas

Diseñar protocolos de separación, purificación y caracterización de moléculas biológicas

Manejar adecuadamente los equipos y el material propio de un laboratorio de bioquímica y biología molecular

Participate in multidisciplinary teams, engaging in teamwork and collaboration.

Propose creative and innovative solutions to complex situations or problems, typical of the area of ¿¿¿¿connection, to donate responses to the various professional and social needs

Que el estudiantado demuestre su capacidad para calcular correctamente los parámetros relevantes de un proceso o un experimento mediante la representación de los datos experimentales

Saber comunicarse de manera efectiva, tanto de forma oral como escrita, adaptándose a las características de la situación y de la audiencia

Ser capaz de diseñar protocolos y utilizar las técnicas del DNA recombinante

Use English to write reports and to interpret information from protocols, manuals and databases.

Work in laboratories, including safety procedures, waste management and accurate activity logging.

DESCRIPTION OF CONTENTS

1. Unit 1. What is the technology of the recombinant DNA?

Historical introduction. The concept of recombinant DNA. The concept of cloning. The impact of recombinant DNA technology: the emergence of molecular biotechnology. Ethical considerations.

2. Units 2,3. General Techniques

Unit 2. GENERAL TECHNIQUES I.

Extraction, purification and analysis of DNA and RNA. Basic enzymology used in DNA manipulation. Restriction enzymes and restriction maps. DNA polymerases, ligases, recombinases and other enzymes of interest.

Unit 3. GENERAL TECHNIQUES II.

Nucleic acid hybridisation: Factors affecting hybridisation, stages of the process and hybridisation methods. Probe labelling: Direct and indirect; types of label; methods for synthesising a labelled probe. Automated oligonucleotide synthesis. Applications of synthetic oligonucleotides. Synthesis of complete genes.

3. Unit 4. Polymerase chain reaction (PCR)

Characteristics of PCR: amplification and specificity. Basic reaction: design of primers. Analysis of the PCR product: cloning and direct sequencing. PCR reverse. Amplification of cDNA: RT-PCR. The PCR as a tool in genetic engineering. Real-Time PCR. PCR applications in other fields. Other amplification systems.

4. Units 5-8. Genetic Engineering

Unit 5. CONSTRUCTION OF CHIMERA DNA. Cloning strategies. Binding of DNA molecules by DNA ligases, DNA topoisomerases and recombination. Introduction of DNA into bacterial cells: transformation and transfection methods.

Unit 6. Cloning vectors in *E. coli*. General characteristics of a vector. Plasmids. Cloning vectors based on plasmids. Phages. Cloning vectors based on phage M13: single stranded vector. Phagemids. Cloning vectors based on phage lambda. Cosmids and vectors for large inserts. Expression vectors in *E. coli*.

Unit 7. Library construction. Genomic libraries. Libraries for sequencing projects. cDNA synthesis methods. Clones with 3 ' ends or 5' of mRNAs. cDNA libraries.

Unit 8. SELECTION OF CLONES. Levels of selection. Identification of recombinant clones. Identification of a specific clone. Direct selection. Selection by immunological techniques. Selection by hybridization with nucleic acid probes.

5. Unit 9: DNA sequencing

Sequencing methods. Automatic sequencing. Strategies for sequencing a DNA fragment.

6. Unit 10. Modification of the DNA sequence

In vivo and in vitro DNA mutagenesis: deletions, insertions and substitutions. Random mutagenesis. Targeted mutagenesis using oligonucleotides. PCR-based targeted mutagenesis techniques, shuffling. Serial mutagenesis. Random and targeted insertion mutagenesis.

7. Unit 11. Methods for the analysis of gene expression

RNA analysis techniques: Northern blot; RT-PCR; RT-qPCR; FISH; use of reporter genes. mRNA mapping. In vitro and in vivo transcription. Translation analysis techniques: In vitro translation; SunTag; polysomes; ribosome profiling.

8. Unit 12. Study of macromolecule interactions

Techniques for studying DNA-protein interactions: Gel mobility shifts; in vitro and in vivo footprinting; chromatin immunoprecipitation (ChIP); purification of DNA-binding proteins: SELEX. Techniques for

studying protein-protein interactions: Purification of protein complexes by TAP; Double-hybrid assay; Co-immunoprecipitation; Pull-down of labelled proteins; Proximity labelling methods; Use of fluorescent proteins for the detection of protein interactions in vivo. Techniques for studying RNA-protein interactions: Gel retardation assay; Chemical modification protection assay; Affinity and UV cross-linking methods: RIP, CLIP, PAR-CLIP.

9. Laboratory classes

1) Construction of a gene library in *Escherichia coli*.

2) Purification of a GST fusion protein.

3) Construction of the restriction map of a plasmid.

4) Design of a CRISPR gene-editing strategy for disruption of the yeast *ADE2* gene.

WORKLOAD

PRESENCIAL ACTIVITIES

Activity	Hours
Theory	29,00
Laboratory	16,00
Total hours	**45,00**

NON PRESENCIAL ACTIVITIES

Activity	Hours
Attendance at other activities	0,00
Individual or group project	17,50
Independent study and work	0,00
Preparation of lessons	20,00
Preparation for assessment activities	30,00
Resolution of case studies	0,00
Total hours	**67,50**

TEACHING METHODOLOGY

The development of the course is structured around theoretical sessions, personal tutorials and practical sessions.

1. Theoretical sessions:

The section on classroom work includes a total of 26 sessions of one hour corresponding to lectures, or

tutorials. Before each class, students will have all the artwork that will be meaningful presented in the website for the Virtual Classroom of the University of Valencia. Thus, it is intended that the student can prepare in advance the classes in order to follow them easier, taking only the notes needed for proper understanding.

2. Personal Tutoring:

The role of tutoring is to help and guide personally the student in all the problems that arise in dealing with the study of the subject. They facilitate the exchange of views between teacher and student, in an effort to approach to individualized instruction.

3. Practical activities:

5 sessions are scheduled with 2-4 hours each (total 16 h). In these sessions several aspects of the techniques of molecular biology and recombinant DNA and other related subjects which are not considered in theoretical sessions and are adaptable to laboratory practice are included.

EVALUATION

Attendance at the laboratory classes is mandatory and so are the resolution of a questionnaire prior to the attendance and the presentation of a report after their realization.

At the end of the course, an exam will be held to assess the knowledge acquired in the theory classes. This exam will consist of two parts: one covering topics 1, 2, 3, 10, 11 and 12, and the other covering topics 4, 5, 6, 7, 8 and 9. To pass, students must achieve a mark of over 5 out of 10 for the exam as a whole and over 4 out of 5 in each of the two parts.

On the final note this exam will have a value of 70%. 20% will correspond to the assessment of practical classes through the memory of practices (1.6 out of 2) and the resolution of the previous questionnaire (0.4 out of 2). The remaining 10% corresponds to activities that will be considered throughout the course.

To pass the subject it is necessary to pass the theory exam (in accordance with the conditions set out above), the practices and the programmed activities.

If the practices or activities were approved but the theory was suspended, the corresponding notes would be saved during the following course to which they have been carried out; from that moment, the practices and / or activities of the continuous evaluation would have to be repeated.

REFERENCES

PRIMROSE S.B. and TWYMAN R.M. (2006). Principles of gene manipulation and Genomics. 7ª ed. Blackwell Publishing.

REAL M.D., RAUSELL, C. and LATORRE, A. (2017). Técnicas de Ingeniería Genética. Ed. Síntesis

GREEN, M.R. and SAMBROOK, J. (2012). Molecular Cloning. A laboratory manual. 4ª ed. Cold Spring Harbor Laboratory Press (3 vols).

AUSUBEL, F.M. et al. (1987-). Current protocols in Molecular Biology. John Wiley & sons.

BROWN, T.A. (2011). Gene cloning and DNA analysis. An introduction. 4ª ed. Ed Blackwell Science.

GLICK, B.R. and PASTERNAK, J.J. (2010). Molecular Biotechnology. Principles and applications of recombinant DNA. 4ª Ed. ASM Press.

GLOVER D. M. and HAMES B.D. (1995). DNA cloning (vol 1, 2, 3, 4). A practical approach. IRL Press.

IZQUIERDO, M. (1999). Ingeniería genética y transferencia génica. Ed. Pirámide.

KREUZER, H. and MASSEY, A. (1996). Recombinant DNA and Biotechnology. A guide for teachers. ASM Press.

LUQUE, J. and HERRAEZ, A. (2001) Biología Molecular e Ingeniería Genética. Harcourt.

PERERA, J., TORMO, A. and GARCIA J.L. (2002). Ingeniería genética. Vol.I. y Vol II. Ed. Síntesis.

WATSON, J.D.; GILMAN, M.; WITKOWSKI, J. and ZOLLER, M. (1992). Recombinant DNA. 2ª ed. Scientific American Books.

WINNACKER E.L. (ed.) (1987). From genes to clones. VCH.

AUSUBEL, F.M. et al. (1987-97). Current protocols in Molecular Biology. John Wiley & sons.

BIRREN ET AL. (1999). Genome analysis. 4 Vols. Cold Spring Harb. Lab.Press.

DIEFFENBACH, C.W. y DVEKSLER, G.S. (1995). PCR primer. A laboratory manual. Cold Spring Harbor.

JAIN, M. (2012) Recombinant DNA techniques. Alpha Science International Ltd. Oxford, U.K.

CLARK, D.P., PAZDERNIK, N.J., MCGEHEE, M.R. (2019) Molecular Biology. 3rd Edition. Academic Press (Elsevier), London.

FOR LABORATORY CLASSES

MINGARRO, G., DEL OLMO, M. (2023). Improvements in the genetic editing technologies: CRISPR-Cas and beyond. Gene 852:147064. doi: 101016/j.gene.2022.147064

SEHGAL, N., SYLVES, M.E., SAHOO, A., CHOW, J., WALKER, S.E., CULLEN, P.J., BERRY, J.O. (2018). CRISPR gene editing in yeast: An experimental protocol for an upper-division undergraduate laboratory course. Biochem. Mol. Biol. Educ. 46:592-601. doi: 10.1002/bmb.21175.

SHORTT, C., KRIPPAEHNE, E., WASKO, B.M. (2023). A simple and accessible CRISPR genome editing laboratory exercise using yeast. microPublication Biology. doi:10.17912/micropub.biology.000699.