

*Genetic Engineering (F.Ciencias) Guía docente 2025-26* 

# PRESENTACIÓN / PRESENTATION

The program addresses the basic principles of the most commonly used methods and techniques in Genetic Engineering and Recombinant DNA Technology, which in turn are the basis for the development of multiple applications in the fields of biomedicine and biotechnology. The aim is that students acquire enough basic knowledge to understand the main methodology and tools currently used in this area so that they will be well prepared to follow its development in the future.

Sin título.jpg

- Titulación: BIOCHEMISTRY
- Módulo/Materia: BIOCHEMISTRY AND MOLECULAR BIOLOGY
- **ECTS**: 6
- Curso, semester: 2nd year, 2nd semester
- Carácter: Obligatory
- Profesorado:
  - Dr. María de Ujué Moreno, mumoreno@unav.es, Ed. Investigación, room 2220
  - Dr. Silvia Cenoz Zubillaga, scenoz@unav.es, Ed. Investigación, room 2111
- Idioma: English
- Aula: 14, Ed. Biblioteca de Ciencias
- Horario: Tuesday 15:00, Wednesday 16:00, Friday 16:00

# RESULTADOS DE APRENDIZAJE (COMPETENCIAS OFICIALES /OFFICIAL COMPETENCIES)

#### Competencias Básicas y Generales

- **CB1:** Que los estudiantes hayan demostrado poseer y comprender conocimientos en un área de estudio que parte de la base de la educación secundaria general, y se suele encontrar a un nivel que, si bien se apoya en libros de texto avanzados, incluye también algunos aspectos que implican conocimientos procedentes de la vanguardia de su campo de estudio.
- **CB2:** Que los estudiantes sepan aplicar sus conocimientos a su trabajo o vocación de una forma profesional y posean las competencias que suelen demostrarse por medio de la elaboración y defensa de argumentos y la resolución de problemas dentro de su área de estudio.
- **CB4:** Que los estudiantes puedan transmitir información, ideas, problemas y soluciones a un público tanto especializado como no especializado.



- **CB5:** Que los estudiantes hayan desarrollado aquellas habilidades de aprendizaje necesarias para emprender estudios posteriores con un alto grado de autonomía.
- **CG1:** Planificar y organizar el tiempo y gestionar la propia formación continua, actualizando el conocimiento de las innovaciones del ámbito científico y saber analizar las tendencias de futuro.
- CG2: Pensar de forma integrada y abordar los problemas desde diferentes perspectivas. Tener razonamiento crítico. Aportar soluciones a problemas en el ámbito científico.
- **CG6**: Trabajar de forma adecuada en un laboratorio con material químico y/o biológico, incluyendo seguridad, manipulación y eliminación de residuos, registro anotado de actividades e interpretación de los resultados.

#### **Competencias Específicas**

- **CE2:** Aplicar las técnicas e instrumentos propios de la experimentación en Bioquímica, Biología y Biología Molecular con seguridad.
- **CE5:** Comprender, analizar críticamente, discutir, escribir y presentar argumentos científicos, tanto en castellano como en inglés, como lengua de referencia en el ámbito científico.
- **CE7:** Comprender bien las diferencias entre los tipos mayoritarios de organismos vivos, desde microorganismos a organismos superiores. Conocer bien la estru y de los tejidos, órganos y sistemas animales y humanos, así como la estructura, variación, función y transmisión del material hereditario.
- **CE9:** Comprender la estructura y función de las bio/macromoléculas, los principales procesos de su transformación y los mecanismos moleculares por los que se regulan, así como los principios que rigen los intercambios de materia y energía con el medio. Conocer las alteraciones moleculares de estos procesos en situaciones patológicas. Conocer las bases y la utilidad de la tecnología del DNA recombinante.
- **CE11:** Conocer los principales temas de debate y retos futuros de la Bioquímica y de la Biología

Molecular, su dimensión social y económica así como sus aplicaciones prácticas.

# COMPETENCIAS ESPECÍFICAS DE LA ASIGNATURA / SPECIFIC COMPETENCIES FOR THIS SUBJECT

#### Knowledge

- Historical background that gave rise to Genetic Engineering and Recombinant DNA Technology.
- Properties and applications of enzymes used in Genetic Engineering. Fundamentals and basic techniques for the isolation and characterization of nucleic acids.
- Fundamentals of the Polymerase Chain Reaction (PCR), as well as its main variants and applications. Methods for DNA and RNA sequencing.
- Types of cloning vectors in eukaryotic and prokaryotic cells and systems for the production of recombinant proteins in both types of cells.
- Methods for the introduction of mutations into genes and proteins and for genome editing.
- Tools and methodologies for the production of Genetically Modified Organisms (GMO) and their applications (transgenic organisms).



• Methods for the analysis of the regulation of gene expression.

#### Skills

- Correct oral and written expression using adequate terminology on the variety of methods, techniques and applications corresponding to the field of Genetic Engineering and Recombinant DNA Technology.
- Ability to interpret correctly a vector map, identifying all the functional elements and all the possible usages for different applications.
- Ability to work properly in a molecular biology laboratory, including the correct handling of samples and very small volumes (μL), safety measures, manipulation and elimination of biological waste and annotated recording of the activities carried out.
- Ability to design experimental strategies for the purification of nucleic acids from biological samples: body fluids, cell cultures, tissues etc. and to determine their concentration and purity.
- Ability to design experimental strategies for cloning, or subcloning from another vector, of a gene fragment or a cDNA starting from total or mRNA.
- Ability to construct the physical and/or restriction map of a DNA sequence by analysis with restriction enzymes.

#### Learning Outcomes

- Demonstrate knowledge, comprehension and practical ability regarding the competencies described, evaluated by the exams and other types of tests performed.
- Demonstrate the capacity to carry out a simple laboratory protocol involving the purification of nucleic acids and the use of enzymes and vectors.
- Demonstrate comprehension of the applications of the methodologies studied in basic research as well as applied to health care, agricultural and food production and industrial sectors.
- Demonstrate having acquired enough training to understand the new advances and the future development of these methodologies.

# PROGRAMA / PROGRAMME

#### THEORY PROGRAMME

#### 1. Introduction: recombinant DNA

Terminology. Historical background. Basics and general scheme of molecular cloning procedure.

#### 2. Enzymes in recombinant DNA technology

Restriction endonucleases. DNA and RNA ligases. Modifying enzymes. DNA polymerases. RNA polymerase. Nucleases. Construction of recombinant DNA molecules.

#### 3. Basic techniques for the manipulation and identification of DNA and RNA

Extraction and purification of DNA, RNA and mRNA. Electrophoresis of DNA and RNA. Transfer of DNA and RNA to membranes (Blotting). Nucleic acids hybridization. Obtaining and labelling DNA and RNA probes.



#### 4. Polymerase Chain Reaction (PCR)

Reaction analysis. Variants: nested PCR, anchored PCR, RT/PCR. Cloning of PCR products. Quantitative PCR. Real-Time PCR.

#### 5. DNA sequencing

Enzymatic method of Sanger. Automatic sequencing. Sanger sequencing strategies. Sequencing databases. RNA sequencing. Next Generation Sequencing: principles and different methods.

#### 6. Prokaryotic cloning vectors

Plasmids: genetic elements, transformation, usage and examples. Phagemids: usage and examples.

#### 7. Eukaryotic cloning vectors

Introduction of DNA into eukaryotic cells: transfection methods. Genetics elements of eukaryotic cell vectors. Shuttle vectors. Expression vectors. Plant cell vectors. Simple virus-derived vectors: retroviruses, adenoviruses, adenovirus-associated virus (AAV).

#### 8. Recombinant proteins

*In vitro* expression of cloned genes. Fusion proteins. Protein expression and production in prokaryotic cells. Protein expression in eukaryotic cells: examples of expression vectors. Purification of expressed proteins

#### 9. Site-Directed Mutagenesis

Random. Punctual. Oligonucleotide directed. Mutant selection. By PCR: in plasmid vectors, in lineal DNA (Overlapping PCR), nested deletions.

#### 10. Genome Editing

Sequence Directed Nucleases (SDN): Meganucleases, Zn-Finger nucleases, TALEN. Natural CRISPR-Cas. CRISPR-Cas9 basic technology, Base Editors, RNA targeting tools, RNA Base Editors, Prime Editing.

#### 11. Genetic inactivation and Genetically Modified Organisms (GMO)

Antisense oligonucleotides. Gene inactivation (Knock Out) in cells. RNA interference. Transgenic (GMO) plants. Transgenic (GMO) animals: tissue-specific and regulated expression. Gene silencing and gene inactivation (Knock Out) in animals. Applications: research, disease models, bioreactors.

#### 12. Methods to study transcriptional regulation of gene expression

Determination of transcription initiation site. Chimeric constructs with reporter genes. Electrophoretic Mobility Shift Assay (EMSA). Chromatin Immunoprecipitation (ChIP).

#### **VECTOR SEMINAR**

Example of the use of a DNA cloning/expression vector



Directional cloning. Use of the different elements of a vector: expression and sequencing.

#### PRACTICALS PROGRAMME

Cloning a fragment of a gene by PCR

# 1. Introductory session: Overview of the experimental strategy that will be followed and explanation of the general plan

#### 2. Isolation and purification of genomic DNA

• Concentration measurement and estimation of purity.

#### 3. Amplification of a gene fragment: genomic DNA and cDNA

- PCR from genomic DNA
- PCR from cDNA provided (RT-PCR)
- Agarose gel electrophoresis analysis

#### 4. Cloning of a gene fragment

- Ligation of purified PCR products provided into a plasmid vector
- Transformation of competent cells
- Plasmid DNA preparation: minipreparation
- Quantification of purified plasmid DNA

#### 5. Digestion with a restriction endonuclease

• Digestion analysis in agarose gel (electrophoresis)

# ACTIVIDADES FORMATIVAS / EDUCATIONAL ACTIVITIES

#### **GENETIC ENGINEERING 6 ECTS (150 hours)**

#### Lectures: 1.52 ECTS (38 hours)

- Presentations by the professor explaining the most important contents of the subject.
- PowerPoint slides will be used throughout the course and made available to the students through ADI.
- Video tutorials and presentations available through the internet explaining different concepts addressed in the lectures will be recommended for the students to watch by themselves
- Several questions will be raised throughout the course for the students to think about and solve at home. They will be addressed in later sessions.

#### Seminar: 0.04 ECTS (1 hour)

• A seminar session will be dedicated to solving an exercise regarding the use of a typical cloning vector and all its elements. This exercise will be given in advance to the students to solve by themselves.

#### Laboratory practice introductory session: 0.04 ECTS (1 hour)



• An introductory session will take place before starting the laboratory work to give an overview of the experimental strategy that will be followed and to explain the general plan. Assistance is **compulsory**.

#### Laboratory practice: 0.62 (15.5 hours)

- **Compulsory** practical laboratory sessions in smaller groups. Some of the methods and techniques studied in lectures will be carried out in the context of a continuous experiment, whose aim is to clone a fragment of a given gene.
- A laboratory manual with the fundamentals of the methods and the working protocols to follow will be made available to the students in advance through ADI. The student must bring the manual to the laboratory every day and must read it in advance.
- A brief quiz will be taken every session, either at the beginning to assess knowledge of the work to be done that day by having read the manual, or at the end to assess the understanding and purpose of the work carried out. These quizzes will have a weight in the final practical score.
- A brief introduction explaining the objectives of the methods to be used will be given at the beginning of each session and the results obtained will be discussed every day in the laboratory.
- Brief videos and animations will be used to demonstrate some laboratory working procedures and technical tips as well as some fundamentals of the methods used
- The aim is that students will be able to follow the working laboratory protocol and carry out the techniques by themselves with the guidance of the teacher and the support of the laboratory assistants.
- After completing all the practical sessions, a written assessment (multiple-choice questions) covering all aspects of the experiments will be carried out. For this test students are allowed to use their laboratory notebook and laboratory manual.

#### Tutorials: 0.02 ECTS (0.5 hours)

• Personal interviews with a professor (tutor) for academic, professional and personal orientation and advice.

#### Personal study: 3.6 ECTS (90 hours)

• Personal study time using the different sources of information proposed.

#### Assessment: 0.16 ECTS (4 hours)

• Different tests to assess both theory and practical knowledge and the acquisition of the defined competencies.

# **EVALUACIÓN / ASSESSMENT**

#### A) FIRST AND SECOND EXAMINATION

1. Practical Sessions: (20% of final mark)



- Attendance at all laboratory sessions, including the introductory session, is **compulsory**. Missing any of the sessions will directly cause not passing the course.
- Daily quizzes (20%).
- Calculations and restriction map (10%).
- Written exam (0.04 ECTS/1 hour) consisting of multiple-choice questions (70%). For this test, students are allowed to use their personal laboratory notebook and the laboratory protocol manual. This exam will be held shortly after the end of the laboratory sessions. In case of not passing, the student will have a second opportunity to pass this written exam at the same time as the final examination in May and a third if necessary in the resit period in June. The daily quizzes and calculation and restriction map marks, obtained before will be maintained with the same weight in the final mark on both occasions.

#### 2. Final Examination (80% of final mark)

- The acquisition of global knowledge and competencies developed in lectures, seminars and laboratory sessions will be evaluated.
- Evaluation will be through a written exam (0.12 ECTS/3 hours) consisting of multiple-choice questions (50%) and short questions (50%). The multiple-choice questions part will be subjected to a reliability analysis that will be taken into consideration to correct the final grades if necessary. A minimum of 4.0 over 10 will be required in each part (multiple choice and short questions) to average them.
- A minimum mark of 4.5 over 10 in this exam (3.6 over 8) will be required to take into account for the final grade calculation the mark obtained in the Practical Sessions or the Continuous assessment. Therefore, passing the subject with a mark of less than 4.5 over 10 in this final written exam will not be possible. In such case, the final grade will be the mark of this written final exam without adding the mark obtained in the Practical Sessions or the Continuous assessment.

#### 3. Continuous assessment (up to an extra 10%)

• Continuous assessment, with quizzes, written assessments and class participation, can earn the students up to an extra 10% in the final mark.

#### 4. Examination of the students in the resit examination period

- A written exam, with the same characteristics and percentage weight in the final mark as the final exam of the ordinary examination period (80%), will be taken.
- The Practical Sessions mark obtained before, with its percentage weight in the final mark (20%), will be maintained if it was enough to pass (5 over 10). If not, a new written multiple-choice questions exam, with the same characteristics as the one taken before, will be taken, having the same percentage weight in the final mark. The daily quizzes and calculations, and restriction map mark obtained before will be maintained with the same weight in the final mark.
- The continuous assessment mark will also be taken into account

#### **B) ADDITIONAL EXAMINATIONS**

1. Third and Fourth Examinations



• With the Practical Sessions passed, the student can choose one of two options, sending an email to the teacher the first week of the semester:

**A:** Keep the Practical Sessions mark from the previous academic year. The evaluation will be the same as for the first examination period students, applying the previous Practical Sessions mark to the total final mark with its respective percentage weight (20%).

**B**: Not to keep the Practical Sessions mark from the previous academic year. The final exam will have a 100% weight in the total final mark.

#### • With the Practical Sessions not passed:

The Practical Sessions must be repeated and the evaluation will be the same as for first examination period students.

#### 2. Fifth examination and thereafter

#### The final exam will account for 100% of the mark

**IMPORTANT:** In all these additional examinations, the students can request, during the **first week** of class, **additional voluntary activities** to count for the extra 10% of the continuous assessment.

# HORARIOS DE ATENCIÓN / OFFICE HOURS

#### Please arrange an appointment by e-mail

#### Dra. Mª de Ujué Moreno Zulategui: mumoreno@unav.es

Department of Biochemistry and Genetics.

Room 2220, 2<sup>nd</sup> Floor Research Building. (*Edificio de Investigación*)

#### Dra. Silvia Cenoz Zubillaga: scenoz@unav.es

Department of Biochemistry and Genetics.

Room 2111, 2<sup>nd</sup> Floor Research Building. (*Edificio de Investigación*)

# **BIBLIOGRAFÍA / BIBLIOGRAPHY AND RESOURCES**

#### Basic (English)

• Primrose, S. B., Twyman, R. M. (2006). Principles of Gene Manipulation and Genomics (7<sup>th</sup> Edition). Blackwell Publishing, Malden, MA. EE.UU. *Text* specialized in Genetic Engineering with a part dedicated to the fundamental tools and methodologies, another part dedicated to the cloning in microorganisms, plants and animals, another part dedicated to genomic and proteomic analysis, and another to the applications in biomedicine and biotechnology. Find it in the Library



• Watson, J. D., Myers, R. M., Caudy, A. A., Witkowski, J. A. (2007). Recombinant DNA. Genes and Genomes A Short Course (3<sup>rd</sup> Edition). W.H. Freeman and Company Cold Spring Harbor Laboratory Press, New York, NY, EE.UU. Contains chapters on the theoretical principles and on techniques and methodologies, reducing and summarizing methods already known for several years and presenting the more recently developed methods and techniques. It also contains chapters on the development of methods for the study of complete genomes and their applications. The graphic illustrations are excellent as in previous editions. Find it in the Library

#### Basic (Spanish)

- Perera, J., Tormo, A., García, J. L. (2002). Ingeniería Genética. Volumen I: preparación, análisis, manipulación y clonaje de DNA. Volumen II: expresión de DNA en sistemas heterólogos. Editorial Síntesis, Madrid, España. Very complete specialized text in Genetic Engineering covering all aspects related to molecular cloning and the expression of cloned genes in different systems. <u>Find it in the</u> Library
- Herráez, A. (2012). Texto Ilustrado e Interactivo de Biología Molecular e Ingeniería Genética (2ª Edición). Conceptos, Técnicas y Aplicaciones en Ciencias de la Salud. Editorial Elsevier España S.L., Barcelona, España. Basic text of Molecular Biology that contains a part with chapters on the theoretical principles, another part with chapters on the more basic techniques and methodology, and another with chapters on the principal applications. Excellent graphic illustrations with explanations included in the figures. Find it in the Library

#### Complementary (English)

- Watson, J. D., Gilman, M., Witkowski, J., Zoller, M. (1992). Recombinant DNA (2<sup>nd</sup> Edition). W.H. Freeman, New York, NY. EE.UU. Introductory text on recombinant DNA technology with chapters on the theoretical principles, chapters on the techniques and methodologies, and chapters on some of the main applications. Excellent graphic illustrations. Although it is a bit out of date in the aspects that more have developed further in this methodology, it is still an excellent introductory text on the main theoretical and practical principles. Find it in the Library
- Thieman, J. T., Palladino, M. A. (2019). Introduction to Biotechnology (4<sup>th</sup> Edition). Pearson Education London United Kingdom. Basic biotechnology text with balanced coverage of basic cell and molecular biology fundamental techniques, historical accounts, new advances and hands-on applications. Information to understand the science and business biotechnology. The 4th edition updates every chapter with the most relevant upto-date changes in technology, applications, ethical issues and regulations. <u>Find</u> it in the Library

#### **Complementary (Spanish)**

• Thieman, J. T., Palladino, M. A. (2010). Introducción a la Biotecnología (2ª Edición). Pearson Education S. A. Madrid, España. Basic biotechnology text with a brief introduction to Molecular Biology and Recombinant DNA technology. Chapters on the different fields of application for biotechnology: vegetable, animal, forensic, environmental, aquatic and medical. Final chapters on ethics. Find it in the Library



Monograph Studies of Cellular and Molecular Biology

- Krebs, J. E., Goldstein, E. S., Kilpatrick, S. T. (2018). Lewin's Genes XII (12<sup>th</sup> Edition). Jones and Bartlett Learning, Burlington MA. EE.UU. Find it in the Library
- Krebs, J. E., Goldstein, E. S., Kilpatrick, S. T., Lewin, B. (2021). Lewin's Essential Genes (4<sup>th</sup> Edition). Jones and Bartlet Learning, Burlington MA. EE.UU. <u>Find it in</u> the Library
- Alberts, B., Johnson, A., Lewis, A., Morgan, D., Raff, F., Roberts, K., Walter, P.
  T. (2015). Molecular Biology of the Cell (6<sup>th</sup> Edition). Garland Science, New York NY. EE.UU. Find it in the Library
- Watson, J. D., Baker, T. A., Bell, S. P., Gann, A., Levine, M., Losick, R. M. (2016). Biología Molecular del Gen (7<sup>a</sup> Edición). Editorial Médica Panamericana. Madrid, España. Find it in the Library
- Watson, J. D., Baker, T. A., Bell, S. P., Gann, A., Levine, M., Losick, R. M. (2014). Molecular Biology of the Gene (7<sup>th</sup> Edition). Pearson Education. Cold Spring Harbor Laboratory Press, New York, NY, EE.UU. Find it in the Library
- Lodish, H., Berk, A., Kaiser, C.A., Krieger, M., Bretscher, A., Ploegh, H., Amon, A., Scott, M. P. (2016). Biología Celular y Molecular (7ª Edición). Editorial Médica Panamericana. Madrid, España. Find it in the Library
- Lodish, H., Berk, A., Kaiser, C.A., Krieger, M., Bretscher, A., Ploegh, H., Amon, A., Martin, K. C. (2016). Molecular Cell Biology (8<sup>th</sup> Edition). W. H. Freeman. S. Francisco CA. EE.UU. Find it in the Library

*Basic texts on Cellular and Molecular Biology with some chapters on methods and techniques related to nucleic acids, proteins, recombinant DNA and Genetic Engineering.* 

#### Laboratory protocols manuals

- Green, M.R., Sambrook, J. (2012). Molecular Cloning. A Laboratory Manual. 4<sup>th</sup> Edition. Cold Spring Harbor Laboratory Press, New York. EE.UU. *Manual with many laboratory protocols that also contains brief introductions on the basic fundaments of each methodology. Find it in the Library*
- Current Protocols in Molecular Biology (1987-2022). Edited by Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.A. and Struhl, K. John Wiley & Sons Inc. Hoboken, NJ, EE.UU. <u>https://currentprotocols.onlinelibrary.wiley.com</u> /journal/19343647
- Current Protocols in Protein Science (1995-2022). Edited by ColiganJ.E., Dunn, B. M., Speicher, D.W. and Wingfield, P.T. John Wiley & Sons Inc. Hoboken, NJ, EE.UU. https://currentprotocols.onlinelibrary.wiley.com/journal/19343663

Collection of laboratory protocols. First published in 1987, Current Protocols in Molecular Biology and Current Protocols in Protein Science established the gold standard for protocol publications. With regularly updated and new material, this extensive collection of protocols ranges from the most basic techniques for isolating and manipulating nucleic acids and proteins to a wide range of advanced and specialized methods updated quarterly. Besides the detailed protocols, they contain brief introductions to the basic fundaments of each methodology.