VNIVERSITAT DE VALÈNCIA

Course Guide
33178 Methods in molecular biology and genetic engineering

COURSE DATA

Data Subject	
Code	33178
Name	Methods in molecular biology and genetic engineering
Cycle	Grade
ECTS Credits	4.5
Academic year	2023 - 2024

Study (s)

Degree	Center	Acad. year	Period
1102 - Degree in Biotechnology	Faculty of Biological Sciences	3	Second term

Subject-matter

Degree	Subject-matter	Character
1102 - Degree in Biotechnology	86 - Cellular and molecular methodology	Obligatory

Coordination

Name	Department
ALEPUZ MARTINEZ, ELIA PAULA	30 - Biochemistry and Molecular Biology
OLMO MUÑOZ, MARCEL.LI DEL	30 - Biochemistry and Molecular Biology

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 (RD 1393/2007) // LEARNING OUTCOMES (RD 822/2021)

1102 - Degree in Biotechnology

- Properly handle the equipment and material of a biochemistry and molecular biology laboratory.
- Saber realizar análisis de expresión génica.
- Be able to use recombinant DNA techniques and design protocols.

LEARNING OUTCOMES (RD 1393/2007) // NO CONTENT (RD 822/2021)

It is intended that after having completed this course, students could know the basic techniques that are used for gene expression studies and manipulation of genetic material.

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.

2. Units 2,3. General Techniques

Unit 2. GENERAL TECHNIQUES I. Basic Enzymology used in the manipulation of DNA. Restriction enzymes and restriction maps. DNA polymerases, ligases, recombinases and other enzymes of interest. Precipitation and purification of DNA. Preparation of plasmids and other DNAs.

Unit 3. GENERAL TECHNIQUES II. Hybridization of nucleic acids: Factors affecting;steps of the process, methods of hybridization. Probes labelling: direct and indirect types of label, methods of synthesis of a labeled probe. Automated synthesis of oligonucleotides. Applications of synthetic oligonucleotides. Complete gene synthesis.

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. Quantitative 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: binding of sticky ends, binding by adding linkers, adapters, and homopolymer tails. Introduction of DNA into bacterial cells: transformation and transfection methods.
Unit 6. Cloning vector 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 . Cosmids and vectors for large inserts. Expression vectors in E. coli: bacterial promoters.
Unit 7. Library construction. Genomic libraries. Libraries for sequencing projects. cDNA synthesis methods. Clones with 3 ' ends or 5' of mRNAs. cDNA libraries. Subtracted 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 seque ncing a DNA fragment.

6. Unit 10. Modification of the DNA sequence

Mutagenesis in vitro of passenger DNA: deletions, insertions and substitutions. Random mutagenesis. Directed mutagenesis using oligonucleotides. Directed mutagenesis techniques based on PCR. Random insertional mutagenesis.

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

Detection and quantification of the transcript. Use of reporter genes. Identification of regulatory elements of transcription. Mapping messengers. Methods for the analysis of individual gene expression. Analysis of differentially expressed genes. Transcription in vitro and in vivo. Translation in vitro.

8. Techniques for studying protein-DNA interactions: gel mobility shift;Unit 12. Unit 12. Study of macromolecule interactions

Techniques for studying protein-DNA interactions: gel mobility shift; Protection to the attack of nucleases and chemical agents in vitro and in vivo; Methylation interference; Cross-linking with UV light. Chromatin immunoprecipitation. Purification of proteins that bind to DNA. Techniques for studying RNA-protein interactions: gel retardation assay; chemical modification protection assay; Methods of affinity; Crosslinking by UV light; Triple hybrid. Techniques for studying protein-protein interactions: Purification of protein complexes, two hybrid, co-immunoprecipitation, Pull-down of tagged proteins; Application of fluorescent proteins for detecting protein interactions in vivo.

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) Obtaining gene-specific probes by PCR

WORKLOAD

ACTIVITY	Hours	% To be attended
Theory classes	29,00	100
Laboratory practices	16,00	100
Preparation of evaluation activities	30,00	0
Preparing lectures	20,00	0
TOTAL	**95,00**	

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, seminars 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:
4-5 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 there will be an exam to assess the knowledge acquired in the theoretical and practical classes. On the final note the theoretical part of 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, 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

Basic

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- GREEN, M.R.y SAMBROOK, J. (2012). Molecular Cloning. A laboratory manual. 4ª ed. Cold Spring Harbor Laboratory Press (3 volúmenes).
- IZQUIERDO, M. (1999). Ingeniería genética y transferencia génica. Ed. Pirámide
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- CLARK, D.P., PAZDERNIK, N.J., MCGEHEE, M.R. (2019) "Molecular Biology". Third Edition. Academic Press (Elsevier), London.

Additional

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

- GLOVER D. M. y HAMES B.D. (1995). DNA cloning (vol 1, 2, 3, 4). A practical approach. IRL Perss
- KREUZER, H. y MASSEY, A. (1996). Recombinant DNA and Biotechnology. A guide for teachers. ASM Press.
- LUQUE, J. y HERRAEZ, A. (2001) Biología Molecular e Ingeniería Genética. Harcourt.
- PERERA, J., TORMO, A. y GARCIA J.L. (2002). Ingeniería genética. Vol.I. y Vol II. Ed. Síntesis.
- WATSON, J.D.; GILMAN, M.; WITKOWSKI, J. y ZOLLER, M. (1992). "Recombinant DNA". 2a ed. Scientific American Books.
- WINNACKER E.L. (ed.) (1987). "From genes to clones". VCH.
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- BIRREN ET AL. (1999). Genome analysis. 4 Volúmenes. Cold Spring Harb. Lab.Press
- DIEFFENBACH, C.W. y DVEKSLER, G.S. (1995). PCR primer. A laboratory manual. Cold Spring Harbor.