Universitat de Lleida

DEGREE CURRICULUM

GENETIC ENGINEERING

Coordination: GARÍ MARSOL, ELOI

Academic year 2021-22

Subject's general information

Subject name	GENETIC ENGINEERING
Code	101516
Semester	2nd Q(SEMESTER) CONTINUED EVALUATION
Typology	

Degree	Course	Character	Modality
Bachelor's Degree in Biomedical Sciences	2	COMPULSORY	Attendance-based
Master's Degree in Biomedical Research		COMPLEMENTARY TRAINING	Attendance-based

Course number of credits (ECTS)	6
Type of activity, credits, and groups	

Activity type	PRALAB		PRAULA	TEORIA
Number of credits	0.5	0.3	2.2	3
Number of groups	3	4	2	1

Coordination	GARÍ MARSOL, ELOI
Department	BASIC MEDICAL SCIENCES
Teaching load distribution between lectures and independent student work	Hours teaching 60 Hours studying 90 Master classes 30 hours Lab practices 8 hours Practical course 22 hours
Important information on data processing	Consult this link for more information.
Language	Catalan for teaching English for protocols Catalan, Spanish and English for questions and tutoring
Distribution of credits	50% theory 50% practice

Teaching staff	E-mail addresses	Credits taught by teacher	Office and hour of attention
GARÍ MARSOL, ELOI	eloi.gari@udl.cat	8,9	
GUASCH VALLÉS, MARTA	martaguasch@cmb.udl.cat	1,2	

Learning objectives

The student must be able to

Demonstrate knowledge of basic concepts and terminology related to the processes of gene isolation, amplification, and manipulation. Lessons 1 to 9. Competences 47, 50, 51 and 60.

Demonstrate knowledge of the basic techniques, methodologies, and processes required to identify, clone, and manipulate a gene. Lessons 1 to 9. Competences 51, 57 and 60.

Demonstrate knowledge about the particularities of biomedical and biotechnological interest of the different groups of living organisms, and especially of the most common host organisms in genetic engineering. Lessons 10 and 11. Competences 51 and 60.

Design and solve simple cloning and mutagenesis strategies. Practices and problems. Competences of the 51, 57 and 60.

Decide between different expression systems and host organisms according to the purpose of the experimental and / or productive process. Practices and problems. Competences 51 and 60.

Competences

CB1 That students have demonstrated possession and understanding of knowledge in an area of study that is based on general secondary education, and is usually found at a level that, although supported by advanced textbooks, also includes some aspects that they involve knowledge coming from the forefront of their field of study.

CB2 That students know how to apply their knowledge to their work or vocation in a professional way and possess the skills that are usually demonstrated through the development and defense of arguments and problem solving within their area of study

CE50. Distinguish the singularities of molecular genetic analysis and its biotechnological and biomedical implications.

CE51. Define the fundamentals and apply the methodology used in the genetic modification of organisms.

CE57. Apply and evaluate electrophoretic methods for the separation of proteins and nucleic acids

CE58 Apply and assess qualitative and quantitative immunological techniques applied to the analysis of molecules and cells.

CE59 Apply luminometry, cytometry, chromatography and spectrometry techniques.

CE60. Apply the basic methods of Molecular Biology used in biomedical research

Subject contents

Part 1. DNA cloning

Topic 1. Isolation and purification of nucleic acids. Cell lysis. Purification of DNA and RNA. Alkaline lysis. Separation of DNA fragments by electrophoresis. Purification of DNA fragments. Pulsed field. 3h

Topic 2 . Recombinant DNA I. Nucleic acid degradation. Nucleases. Restriction endonucleases. Nucleic acid synthesis. Polymerases. Modification of nucleic acids. Ligase. 2h

Topic 3- Recombinant DNA II. Plasmids and vectors. Methods for cloning. Selection of recombinant clones. Cloning strategies. Fill-in. Linkers. Gene libraries. 3h

Topic 4- Polymerase Chain Reaction. Heat resistant polymerase. Stages and PCR reaction. Design of primers. DNA polymerase features. PCR efficiency. Reverse transcriptase PCR and RT. PCR cloning. Real time PCR. Digital PCR. 4h

Topic 5- PCR Cloning. TA cloning. Gibson Assembly. Topo cloning. Gateway cloning. Recombinant cloning. 2h

Topic 6- Transformation of DNA. Vectors. Protocols of transformation in bacteria and yeast. Vectors with broad spectrum of host. Transfection protocols. Lentiviral vectors. 3h

Topic 7- Genetic manipulation. Manipulation of bacteria and yeast. Gene tagging. RNAi. Crispr-Cas technology. Knock out. KO conditional. Knock in. GMOs. Gene therapy. 4h

Topic 8- Expression vectors. Features of an expression vector. Inducible promoters. Gene reporters. In vitro transcription and translation. 2h

Topic 9- Production of heterologous proteins. Conditions for maximum expression and production. Codon usage. Glycosylation. Protein purification. Secretion Vectors. Production in transgenic organisms. 2h

Part 2. Molecular Biology Techniques

Topic 10- Gene synthesis. Oligonucleotide synthesis. Gene synthesis. Genome synthesis. 2h

Topic 11- DNA sequencing. Next Generation Sequencing. Solid-Bridge and emulsion. Ion Torrent. 3h

Problems and examples

1. Quantification and structure of nucleic acids. Calculating the concentration of nucleic acids designed for ligation reactions, phosphorylation and ends-modification. Restriction mapping, Genetic and physical maps. 6h

2. Cloning strategies. Having different genes and vectors, the student have to design protocols to clone DNA. Selection of the best strategy. 6h

3. Design of PCR primers. Primers for gene amplification. Calculations of the melting and anneling temperatures. PCR conditions. 6h

4- Strategies for site-directed mutagenesis. Learning primers design to produce different types of mutations. 4h

Practical courses

Practice 1- Purification of DNA fragment from an agarose gel. Electrophoresis of digested DNA and recovery of a fragment from agarose gel. Columns of silica gel. Finally check purification. 5h

Practice 2 Purification of RNA from human biopsies. Determination of RQI. In collaboration with the Tissue

Bank (biobank) of IRBLleida, total RNA is obtained from frozen biopsies by using the robotic system, Maxwell® 16 LEV simplyRNA Tissue Kit Promega. Finally, the students evaluate the integrity of the RNA extracted measuring the quality of RNA (RQI) with a device from BioRad Experion. 3h.

Methodology

To achieve the objectives and acquire the skills will be scheduled the following activities:

- Lectures. (CM)

These will be conducted with all students. The main goal is to give an overview of the thematic content of the methodologies and techniques.

- Problems. (Pro)

We provide to the students a list of quantification, cloning, primers design and mutagenesis problems. Students must solve these problems which will serve as a model for the exam questions.
The problems have as a main objective that the students implement the technical concepts and design methodologies in real situations in the laboratory.

-Laboratory. (PL).

The labs are intended for students to relate the theoretical aspects of the methodologies with practical protocols for different products and kits used in the molecular biology laboratory. These protocols are always in English so students should have a basic level of English. These practices will be conducted in small groups.

Development plan

Theory. Attended

Part 1. Recombinant DNA technology. Topics from 1 to 9. 25 hours. Professor Eloi Garí

Part 2. Techniques of Molecular Biology. Topics from 10 to 11. 5 hours. Professor Eloi Garí

Problems. Attended

Quantification and structure of nucleic acids. Cloning procedures. Design of primers and PCR. Site-directed mutagenesis. 22 hours. Professor Eloi Garí. Two groups.

Practices. Attended

Practice 1- Purification of a DNA fragment from an agarose gel. 5 hours. Professor Eloi Garí. Three groups.

Practice 2- RNA purification of human biopsies. 3 hours. Professor Eloi Garí. Four groups of 10 students maximum.

It is **MANDATORY** that students bring in the course of teaching practices:
White-lab coat
Safety glasses
Chemical protection gloves

GENERAL SAFETY RULES IN LABORATORY PRACTICES
- Maintain the place of performance of clean and tidy practices. The work table must be free of backpacks, folders, coats ...

- In the laboratory you can not come with shorts or short skirts.
- Bring closed and covered shoes during the performance of the practices.
- Bring long hair always collected
- Keep the gowns cords to protect against spills and spills of chemical substances.
- Do not wear wide bracelets, pendants or sleeves that can be trapped by the equipment.
- Avoid wearing contact lenses, since the effect of chemicals is much greater if they are introduced between the contact lens and the cornea.
- Do not eat or drink in the laboratory
-Smoking is forbidden within laboratories
-Wash your hands whenever you have contact with a chemical and before leaving the laboratory.
-Follow the teacher's instructions and consult any questions about security

Evaluation

Evaluation- Exams		
	% of final score	Exam model
Theory	40 10	Test Short questions format
Practices	10	Short questions format
Problems	40	Solving problems and cases

There will be three exams:

Evaluation of the work in the laboratory using short questions to assess the student's ability to understand the protocols used in practice. This evaluation will represent 10% of the final grade.

Assess the knowledge of students with the theoretical concepts given in the first part of the course. There will be a multiple choice assessment for the chapters 1 up to 9th. This evaluation will represent 40% of the final grade. For chapters 10 to 11 the evaluation will be in format of short questions counting a 10% of the final score.

Finally, we will assess the ability to solve problems and cases in the field of basic genetic engineering. There will be a written assessment in the form of problem solving cases. This evaluation will represent 40% of the final grade.

To pass the partial exams the course students have to obtain a global score of 60% for the theory and problems exam. They can rescue the exams in the final exam obtaining 50% in the global score.

Bibliography

Basic

-“**Cálculo en Biología Molecular y Biotecnología.** Guía de matemáticas para el laboratorio”. 2ª Edició. 2012. F.H. STEPHENSON. Academic Press. Ed. Elsevier

-“**Molecular Biotechnology**” 3ª Edició. 2003. BR. GLICK, JJ. PASTERNAK. ASM Press.

-“**Principles of Gene Manipulation**” 6ª Edició. 2001. SB. PRIMROSE, RM. TWYMAN, RW. OLD. Blackwell Science Publishers.

-“**Gene Cloning *and DNA analysis. An Introduction***” 4ª Edició. 2001. TA. BROWN. Blackwell Science Publishers.

-“**Ingeniería Genética y Transferencía Génica**” 1ª Edició. 1999. M. IZQUIERDO ROJO. Ed. Pirámide.

-“Ingeniería Genética. Vol I i II” 1ª Edició. 2002. J. PERERA, A. TORMO i JL. GARCIA. Ed. Síntesis

-"Tècnicas de Ingeniería Genética". 1ª Edició. 2017. MD. REAL GARCÍA, C. RAUSELL i A. LATORRE. Ed. Síntesis.

- **The Crispr page at CNB**. http://wwwuser.cnb.csic.es/~montoliu/CRISPR/

Complementary

-“**Current Protocols in Molecular Biology**” Edició en CD rom. 2006. John Wiley Press.

-“**Molecular Cloning.** ***A Laboratory Manual***” 3ª Edició. 2001. J. SAMBROOK, DW. RUSSELL. CSHL Press.

-“**Biotechnologie**” 5ª Edició. 1999. R. SCRIBAN (coord). Ed. Tec&Doc.

-“**DNA Science**. *A First Course*.” 2ª Edició. 2003. DA. MICKLOS, GA FREYER. CSHL Press.

-“**Genes VIII**” 8ª Edició. 2004. B. LEWIN. Prentice Hall.

- “**Biología Molecular e Ingeniería Genética.** Conceptos técnicas y aplicaciones en ciencias de la salud”. 2ª Edició. 2012. A. HERRÀEZ. Ed. Elsevier