

**COURSE DATA****Data Subject**

Code	33182
Name	Transgenic organism acquisition
Cycle	Grade
ECTS Credits	4.5
Academic year	2022 - 2023

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
ARRILLAGA MATEOS, ISABEL	25 - Plant Biology
OLMO MUÑOZ, MARCEL.LI DEL	30 - Biochemistry and Molecular Biology

SUMMARY

This course will provide the scientific basis and methodology used to obtain genetically modified organisms (GMOs), particularly fungi, yeasts, plants, invertebrates and mammals. The aim of the practical part of the course is to teach the students some of the techniques most commonly used in laboratories that produce GMOs.

PREVIOUS KNOWLEDGE**Relationship to other subjects of the same degree**

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



Other requirements

To take this course you need to have studied or be studying the course Methods in Molecular Biology and Genetic Engineering

OUTCOMES

1102 - Degree in Biotechnology

- Saber diseñar y construir un organismo transgénico.

LEARNING OUTCOMES

Knowledge of the basic principles and methodology of genetic transformation in different organisms.
Understanding and managing basic scientific terminology related to the subject.
Knowing how to find the appropriate literature for being able to update and increase their knowledge on a specific topic
Know how to apply basic techniques related to the subject
Understanding and interpreting scientific papers related to the subject.
Select strategies for handling a specific problem.
It is intended that, at the completion of the course, the student will be able to communicate the contents of the subject and to discuss and argue about matters of scientific interest using the contents of this subject. Particularly:
Ability to work in groups in order to cope with problematic situations collectively.
Ability to argue from rational criteria, distinguishing between thoughts or feelings and accepted scientific evidences.
Ability to speak to a public audience, such as the class itself, by exposing a brief work or to participate in a discussion about a controversial topic or issue.
Ability to interact with both the teacher and the other students.
Ability to build a comprehensive and organized text.
Acquisition of social and professional awareness about environmental issues and the importance of biotechnology and its ethical implications.

DESCRIPTION OF CONTENTS

1. Introduction

Basic features about the generation of transgenic organisms



2. Yeast and Fungi

1. Genetic modification of yeast and fungi used in biotechnology. Biotechnological importance of genetic manipulation of yeast and fungi. Cloning in the yeast *Saccharomyces cerevisiae*: development of vectors, selection markers, introduction of permanent modifications by specific recombination (deletions, changes of promoters and tagged proteins), transformation and verification by PCR of the transformants. Cloning in non-*Saccharomyces* yeasts. Manipulation of filamentous fungi. Examples of some genetic manipulations in yeast and fungi of biotechnology relevance (improved efficiency in alcoholic beverages production and use of yeasts and fungi as factories).

3. Virus, gene therapy.

2. Gene therapy and genetic modification of viruses. Viruses as carriers of genes: potential applications in human health. How to convert a virus in a vector. General properties of viruses used as vectors: Retrovirus, Lentivirus, Adenovirus, Adeno-associated virus (AAV), Herpes simplex virus. How to combine properties of more than one virus.

3. Defective Non-replicating viral vectors. Retrovirus and lentivirus vector non-replicative. Gene therapy of severe combined immunodeficiency (SCID) with a modified retrovirus. Adenovirus vectors of non-replicative. Clinical applications. Other viruses such as non-replicating viral vectors.

4. Replicating viral vectors. Oncolytic viruses. Replicative oncolytic adenovirus. Herpes simplex 1 (HSV1) replicative oncolytic. Other viruses as vectors for viral replication. Vaccinia virus-derived vectors.

5. Redirectioning of viral vectors. Retrovirus and lentivirus redirectioning. Adenovirus redirectioning via genetic modification. Adenovirus redirectioning by chemical modification. Redirectioning of other viruses.

4. Transgenic plants

Transgenic plants

6. Introduction. Traditional breeding versus transgenesis. Methods for introducing foreign DNA in plants. Requirements: In vitro propagation of plants, vectors.

7. *Agrobacterium*-mediated transformation (*A. tumefaciens* and *A. rhizogenes*). Methodology and factors affecting the efficiency of transformation.

8. Transformation by DNA gun. Methodology and factors affecting the efficiency of transformation. Other methods.

9. Characterization of transgenic plants. Transient expression, stable integration. Major applications of transgenic plants.



5. Invertebrates

10. Genetic modification of invertebrates: *Drosophila* and *Caenorhabditis elegans*.

Early development and life cycle of *Drosophila*. Transgenesis in *Drosophila*: use of transposable elements as transformation vectors, phenotypic markers, microinjection into the germline of embryos, selection of individual transformants. Random or targeted insertion of transgenes. Life cycle of *C. elegans*. Transgenesis in *C. elegans*: vectors, microinjection vs. ballistic transformation, selection of individual transformants. Applications of transgenesis in *Drosophila* and *C. elegans* for the study of developmental processes and the generation of biomedical models.

6. Mammals

11. Fundamentals of genetic modification in mammals. Generation of transgenic mammals by injection of pronuclei. Fundamentals of reproduction in mammals. Methodology. Design of the transgenes. Use of promoters. Reporter genes. Classic and inducible transgenic animals. Applications of transgenesis in mammals.

12. Mammalian genetic modification using homologous recombination techniques. Fundamentals of early development of mammals and embryonic stem cells. Embryonic stem cell modification. Classic knockouts. Methodology. Knockins. Conditional / tissue-specific and inducible mutants.

13. Introduction of CRISPR methodology in mammals. Origins and historical perspective. Applications for the generation of knockin and knockout mice. Fine genetic editing and modifications that do not affect the gene sequence. Future perspectives of the use of CRISPR technology.

14. Transgenesis in somatic cells in vivo: topical transgenics. Fundamentals of in utero electroporation technique. Cellular and zone specific transgenesis, multiple transgenesis, functional experiments, electroporation using CRISPR tools. iGonad.

7. Laboratory classes

Informatic class work

1. The transgenic Fly Lab (Howard Hughes Medical Institute)

http://www.hhmi.org/biointeractive/vlabs/transgenic_fly/index.html

It is a computer simulation of the process of generating transgenic flies. Protocol is developed sequentially, and some experiments of microinjection of specific constructs are suggested, whose results should be interpreted.

Lab experiments

1 - Disruption of a gene in a haploid strain of *Saccharomyces cerevisiae*.

2 - Assays of transient expression in plant tissues

3 - Analysis of reporters in transgenic mice

**WORKLOAD**

ACTIVITY	Hours	% To be attended
Theory classes	31,00	100
Laboratory practices	12,00	100
Computer classroom practice	2,00	100
Development of group work	16,50	0
Preparation of evaluation activities	25,00	0
Preparing lectures	26,00	0
TOTAL	112,50	

TEACHING METHODOLOGY

The teaching of this subject is based on several educational activities. In the lectures the teacher explains the theoretical basis of the subject. Practices and computer lab allow students to carry out real or virtual activities related to the contents of the course. The seminars allow a deeply understanding of some aspects. In all these activities active participation of students is intended.

EVALUATION**The evaluation of the course will be in two parts:**

Block 1: Review Theoretical / practical. It will consist of a written test that will count up 9 points of the final grade.

Block 2: Includes the evaluation of seminars, workshops and / or memories of practice. This will be done individually or in groups (depending on number of students). It will have up to 1 point of the final grade.

In order to be evaluated is essential to have attended practices, given its mandatory.

To pass the subject must have passed both blocks.

The parties approved of block 2 will be saved during the same academic year and the next one.

REFERENCES**Basic**

- Benítez-Burraco A (2005) Avances recientes en Biotecnología Vegetal e Ingeniería Genética de Plantas. Reverté, Barcelona.
- Brown, T.A. (2004) Gene cloning and DNA analysis: an introduction. 5th ed. Blackwell Science, Oxford.
- Izquierdo-Royo, M. (1999) Ingeniería Genética y transferencia génica. Pirámide, Madrid.
- Parekh S.R. (ed.) (2004) The GMO Handbook. Genetically modified animals, microbes and plants in Biotechnology. Humana Press Inc., New Jersey.



Primrose, S.B., Twyman, R. (2006) Principles of genetic manipulation and genomics. 7th ed. Blackwell Science, Oxford.

Singer, M. y Berg, P. (1993) Genes y genomas: una perspectiva cambiante. Omega, Barcelona.

Slater A, Scott N, Fowler M (2008). Plant Biotechnology. The genetic manipulation of plants. Oxford University Press, Oxford

Hogan BLM, Beddington RSP, Costantini FL (1994) Manipulating the mouse embryo. A laboratory manual. Cold Spring Harbor, NY.: Cold Spring Harbor Laboratory Press.

Additional

- Ashburner, M., Golic, K.G., Hawley, R.S. (2005). Drosophila: A Laboratory Handbook, Second Edition. Cold Spring Harbor Laboratory Press, New York.
- Bhojwani SS, Razdan MK (1996). Plant Tissue Culture: Theory and Practice, a Revised Edition. En: Studies in Plant Science 5. Elsevier, Amsterdam.
- Carroll D.J. (2008). Microinjection: Methods and Applications (Methods in Molecular Biology). Humana Press Inc., New Jersey
- Dahman C. (2008). Drosophila: Methods and Protocols (Methdos in Molecular Biology). Humana Press Inc., New Jersey.
- George EF 1993 Plant Propagation by tissue culture.(Parts I and II) 2nd ed. Exegetics Ltds England
- Murray DR (2003) Seeds of concern. The genetic manipulation of plants. CABI Publishing, Wallingford.
- Potrykus I, Spangerberg G 1995 gene transfer to plants. I Potrykus and G Spangerberg (eds.) Springer- verlag Berlin

Paginas web

- <http://croptechnology.unl.edu>
- <http://www.isaaa.org>
- http://www.hhmi.org/biointeractive/vlabs/transgenic_fly/index.html
- <http://www.jove.com/index/details.stp?ID=833>
- <http://www.wormbook.org>
- <http://www.currentprotocols.com>
- https://web.mit.edu/comp-med/Restrict/CAC/training_new.htm
- <http://www.jax.org/courses/events/current.do>