Universitat Autònoma de Barcelona

2020/2021

Genetically Modified Animals

Code: 100937
ECTS Credits: 6

Degree	Type	Year	Semester
2500253 Biotechnology	OT	4	0

The proposed teaching and assessment methodology that appear in the guide may be subject to changes as a result of the restrictions to face-to-face class attendance imposed by the health authorities.

Contact

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Use of Languages

Principal working language: catalan (cat)
Some groups entirely in English: No
Some groups entirely in Catalan: Yes
Some groups entirely in Spanish: No

Teachers

Miguel García Martínez
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Ivet Elias Puigdomenech
Verónica Jiménez Cenzano

Prerequisites

There are no prerequisites to attend this course. However, to facilitate the student's understanding of the subject matter and the achievement of the learning goals proposed, it is advisable that the student has previous knowledge on Cellular Biology, Genetics, Molecular Biology and Recombinant DNA technology.

It is also advisable that the students have basic knowledge of English, so that they can use the information sources of the field, which are mostly in this language.

Objectives and Contextualisation

The objective of the subject "Transgenic animals" is to provide the students with up-to-date knowledge in transgenesis and related technologies. Thus, the content of the subject will cover the following topics: Description and classification of transgenic animal models; Study of the different methodologies employed to obtain transgenic animal models of different species, and technologies that allow the overexpression of genes or the blockage or modification of endogenous genes, either ubiquitously or in a tissue-specific and/or inducible manner; Establishment and management of transgenic animal colonies; Cryopreservation of embryos and sperm, IVF, Health rederivation, Ethical aspects related to the generation and utilization of transgenic animals; Legislation on the use of laboratory animals; Application of animal transgenesis to the fields of biomedicine, biotechnology and livestock breeding.

Competences

- Adopt clear, objective scientific criteria in order to project a positive, transparent image of biotechnology to economic, political and social agents.
- Apply general laboratory security and operational standards and specific regulations for the manipulation of different biological systems.
- Apply the criteria for evaluating biotechnological risks.
- Apply the principal techniques for the use of biological systems: recombinant DNA and cloning, cell cultures, manipulation of viruses, bacteria and animal and plant cells, immunological techniques, microscopy techniques, recombinant proteins and methods of separation and characterisation of biomolecules.
- Comply with ethical principles and legislation in the manipulation of biological systems.
- Describe the molecular, cellular and physiological bases of the organisation, functioning and integration of living organisms in the framework of their application to biotechnological processes.
- Design and implement a complete protocol for obtaining and purifying a biotechnological product.
- Design continuation experiments for problem solving.
- Display an integrated vision of an R&D&I process, from the discovery of the basic knowledge and the development of applications to market launch, and apply the main concepts of organisation and management to a biotechnological process.
- Identify the strategies for producing and improving products in different sectors using biotechnological methods and display an integrated vision of the R&D&I process.
- Interpret experimental results and identify consistent and inconsistent elements.
- Learn new knowledge and techniques autonomously.
- Make an oral, written and visual presentation of ones work to a professional or non-professional audience in English or in one's own language.
- Make decisions.
- Read specialised texts both in English and ones own language.
- Reason in a critical manner
- Search for and manage information from various sources.
- Search for, obtain and interpret information from the principal databases on biology, bibliography and patents and use basic bioinformatic tools.
- Think in an integrated manner and approach problems from different perspectives.
- Understand the legislation that regulates intellectual property in the area of knowledge and application of biotechnology.
- Use ICT for communication, information searching, data processing and calculations.
- Use the fundamental principles of mathematics, physics and chemistry to understand, develop and evaluate a biotechnological process.
- Work individually and in teams

Learning Outcomes

1. Apply different strategies to optimise the generation and study of genetically modified animals (mouse strain, methodology to apply, organisation of the animal colonies and phenotyping analyses to be performed in each generation/age).
2. Apply operational and safety rules for laboratories and for manipulation of animals in order to perform phenotyping experiments.
3. Describe the approaches to the phenotyping of transgenic animals.
4. Describe the composition of the different types of DNA constructs used to generate genetically modified animals: conventional transgenic, knock-out, knock-in and gene trap
5. Describe the ethical principles and current legislation that apply to animal experimentation and genetic manipulation.
6. Describe the methodologies for generating transgenic and clonal animal models, and their limitations.
7. Design and obtain models of transgenic animals in response to specific biomedical, biotechnological or livestock needs.
8. Design continuation experiments for problem solving.
9. Enumerate the advantages and disadvantages of transgenesis for specific biomedical and biotechnological applications.
10. Evaluate the biotechnological risks in genetic manipulation and in the vectors used.

11. Explain the applications of transgenic animals to biomedicine (basic sciences, obtaining animal disease models, bioreactors, xenotransplantation) and livestock farming, and design models of genetically modified animals for these applications.
12. Explain the different methodologies of use for obtaining transgenic and cloned animals.
13. Explain the fundamental physicochemical principles of transgenesis.
14. Explain what a patent is, its usefulness, and current laws on transgenic animals.
15. Identify the fundamental biochemical principles of transgenesis.
16. Interpret experimental results and identify consistent and inconsistent elements.
17. Learn new knowledge and techniques autonomously.
18. Make an oral, written and visual presentation of ones work to a professional or non-professional audience in English or in one's own language.
19. Make decisions.
20. Read specialised texts both in English and ones own language.
21. Reason in a critical manner
22. Search for and interpret scientific articles on transgenesis in order to design the generation of models of transgenic animals.
23. Search for and manage information from various sources.
24. Think in an integrated manner and approach problems from different perspectives.
25. Use ICT for communication, information searching, data processing and calculations.
26. Use these techniques for obtaining transgenic animals.
27. Work individually and in teams

Content

Knowledge on the following topics will be imparted during the theoretical classes:

TOPIC 1

Introduction to the technologies used for animal genetic engineering. Transgenic animals: definition and classification. Advantages of the mouse as an animal model in biomedicine.

TOPIC 2

Generation of transgenic animals by transgene addition. Preparation of DNA constructs or transgenes. Collection of embryos. Microinjection of DNA into the pronucleus of 1-cell embryos. Transfer of engineered embryos to receptor females. Genotyping of genetically engineered animals. Integration and vertical transmission of the transgene. Mosaic animals. Transgene expression and phenotype.

TOPIC 3

Design and production of chimeric genes/transgenes: promoters, inducible systems, insulators, enhancers. Analysis of transgene expression *in vitro*: technologies for the introduction of exogenous DNA to cultured cells. Transient and stable transfections. BACs and YACs.

TOPIC 4

Generation of transgenic livestock. Introduction of new traits of interest for livestock breeding. Biotechnological applications. Production of proteins with pharmaceutical interest in the mammary gland. Transgenic animals for xenotransplantation.

TOPIC 5

Generation of transgenic animals using viral vectors (lentivirus). Generation of transgenic animals from sperm.

TOPIC 6

Targeted mutagenesis in animals through Embryonic Stem cells (ES cells): definition of ES cells, properties, obtainment and culture. Reprogramming and *Induced Pluripotent Stem cells (iPS cells).*

TOPIC7

Generation of of Knockout / Knockin mice by Gene targeting in ES cells. Design of recombination vectors. Homologous recombination. Selection of recombined ES clones.

TOPIC8

Generation of mouse chimerasdocument injection of recombinant ES cells in blastocytes, injection/ aggregation of 8-cell embryos, injection/ aggregation of tetraploid embryos. Homozygous and heterozygous Knockout / Knockin animals. Applications.

TOPIC 9

Conditional Knockout / Knockin animals: Recombinases systems (*Cre-LoxP, FLP-Frt*). Tissue-specific Knockout / Knockin animals. Inducible Knockout / Knockin animals; inducible systems, transcriptional and post-transcriptional control. Advantages and limitations. Applications.

TOPIC 10

Gene Trap for random mutagenesis. Technology and vectors for Gene Trap. Applications.

TOPIC 11

Use of transposons for obtaining transgenic animals.

New technologies: Generation of Knockout / Knockin animals though genome editing using *Zing Finger Nucleases, TALENs* o *CRISPR-Cas9*. Advantages and limitations. Applications.

TOPIC 12

Generation of clonic animals: Nuclear transfer. Technological aspects and biological implications of nuclear transfer. Reprogramming. Applications. Advantages for the obtainment of transgenic livestock. Therapeutic cloning.

TOPIC 13

Establishment and maintenance of genetically modified mouse and rat colonies. Nomenclature. Phenotype: alterations arising due to transgenesis technology, environmental factors or genetic background.

TOPIC 14

Technologies to support the establishment and the management of colonies of genetically modified animals: Cryopreservation of embryos and sperm. *In vitro* fertilization (IVF). Health rederivation. Ovary transfer.

TOPIC 15

Housing and handling of transgenic animals. Current legislation on animal genetic engineering and use of laboratory animals.

TOPIC 16

Ethical aspects. Ethics committees on animal experimentation. Social Impact. Intellectual property.

TOPIC 17

Large International consortia on mouse mutagenesis. Large-scale phenotyping centres: "Mouse Clinics".

TOPIC 18

Obtainment of transgenic fish. Applications in Biotechnology.

TOPIC 19

Use of transgenic animal models for the study of diseases (I): Diabetis mellitus. Obesity. Use of transgenic animal models for the development of new gene therapy products for these diseases.

TOPIC 20

Use of transgenic animal models for the study of diseases (II): Cancer. Study of oncogenic and anti-oncogenic genes in transgenic animals.

TOPIC 21

Use of transgenic animal models for the study of diseases (III): Models of inheriteddiseases.

TOPIC 22

Use of transgenic animal models in neurosciences. Use of transgenic animal models in the field of immunology.

The laboratory practice classes will cover the design of different types of transgenic animals and Knockout / Knockin mutants, the establishment and maintenance of colonies of transgenic mice and the genotypic analysis of the genetically engineered animals. Students will also carry out several techniques as part of the phenotypic analysis of genetically engineered mice. Using a transgenic mouse model, an *in vivo* phenotyping study will be performed.

Content of the laboratory practice classes:

- Generation of transgenic and Knockout / Knockin animals. Videos.

- Design of transgenes, gene targeting recombination vectors and components of the CRISPR/Cas9 system.

- Handling and *in vitro* culture of pre-implantational embryos.

- Genotype analysis. Establishment of colonies of transgenic animal and Knockout / Knockin mutants.

- Phenotype analysis. Histopathology, necropsy and *in vivo* studies.

Methodology

The subject "Transgenic Animals" consists of theory and laboratory classes, and tutored oral presentations of relevant literature. The formative activities of the subject are complementary.

Theoretical classes

The contents of the theoretical classes will be imparted by a Professor in a series of master classes supported by audio-visual material. The slides used by each professor in each class will be available to the students through the subject'sCampus Virtual/Moodle. These master classes will constitute the main form of transfer of theoretical contents. Students are advised to periodically consult the books and links suggested in the Bibliography section of this document and at the Campus Virtual/Moodle to consolidate and clarify, if necessary, the contents explained in class.

Laboratory practice classes

The laboratory practice classes have been designed to help students get familiarized with the methodologies used to produce transgenic animals, establish animal colonies, genotype genetically engineered animals, and design and perform different phenotypic analyses in these animal models. We expect that, during these laboratory practice classes, students will be able to experience a "real world" situation in which they need to design an experiment, obtain a genetically engineered animal model and study *in vivo* their phenotype. We would like students to experience the excitement associated to the research that uses the technology of animal transgenesis.

The laboratory practice classes are composed of 3 sessions of 4 h each (from 3PM to 7PM), during which students will work in groups of 2-3 people under the supervision of an experienced professor. The date assigned to each laboratory practice group will be published in the subject'sCampus Virtual/Moodle with sufficient anticipation.

Attendance to laboratory practice classes is mandatory.

By the end of the laboratory practice classes, students will need to have answered a questionnaire. Both the laboratory practice guide and the questionnaire will be available through the Campus Virtual/Moodle. Students must bring their own lab coat, a waterproof marker and the Laboratory Practice Guide to each laboratory practice class.

Oral presentations of selected papers

Students will analyse and discuss in an oral presentation in front of the whole class a selected scientific publication on animal transgenesis, published in a recognized international scientific journal. To this end, students will pair with a fellow classmate. During the process of analysis of the paper's content and preparation of the oral presentation, students will be tutored by researchers with experience in the field of animal transgenesis. Students will have 10 minutes for the oral presentation, equally divided amongst the members of the group, plus 5 minutes for questions (total of 15 minutes). The objective of this evaluating activity is that students get used -under the supervision of a tutor- to the process of searching, reading and understanding of scientific literature, and if necessary, develop a critical view on the figures, tables and results described in the publication. On the other hand, with this activity students will increase their knowledge of the current applications of the animal transgenesis technologies.

Tutoring

The oral presentations of selected papers will be tutored. In addition, upon request from the students, individualized tutoring will be available throughout the course. The objective of this sessions will be to help the student resolve doubts and reviewbasic concepts and to provide them with advice on sources of information and the best way to discuss scientific results in public.

Activities

Title	Hours	ECTS	Learning Outcomes
Type: Directed			
Oral presentations	8	0.32	25, 23, 22, 4, 3, 6, 9, 11, 12, 18, 15, 20, 24, 19, 21, 27, 26
Practical lessons	12	0.48	2, 23, 5, 6, 8, 7, 16, 24, 19, 21, 27, 26
Theorical lessons	35	1.4	2, 10, 23, 5, 4, 3, 6, 7, 9, 13, 11, 12, 14, 15, 20, 26
Type: Supervised			
Tutorials	5	0.2	25, 23, 22, 4, 3, 6, 9, 11, 12, 18, 15, 20, 24, 19, 21, 27, 26
Type: Autonomous			
Individual study time	74	2.96	25, 10, 23, 22, 5, 4, 3, 6, 8, 13, 11, 12, 14, 15, 16, 20, 24, 19, 27, 26
Oral presentations	10	0.4	25, 23, 22, 4, 3, 6, 12, 18, 15, 16, 20, 24, 19, 21, 27, 26

Assessment

To pass the course, students must achieve a final score of 5 points (over a total of 10 points) and must attend the laboratory practice classes. The evaluation activities are:

1.- Final examination of theoretical classes

Accounts for 50% of the final score (5 points out of 10). Assessment will consist of a written examination, under the format of a True or False test, on topics explained during the theoretical classes. A score greater than 2.5 in this examination is required to pass the course.

There will be a Second Chance/Recovery Exam, under the same format as the original exam.

2.- Examination of Laboratory classes

Accounts for 15% of the final score (1.5 points out of 10). Assessment will consist of a written examination, under the format of a True or False test, on topics explained during the practical classes. It will be held at the end of practical classes period.

Attendance to practical sessions (or field trips) is mandatory. Students missing more than 20% of programmed sessions will be graded as "No Avaluable.

3.- Self-study exercise

Accounts for 10% of the final score (1 point out of 10). Assessment will consist of an exercise that the student will have to develop on their own. Details will be posted online in the "Campus Virtual" at the end of April.

4.- Oral presentations of selected research papers

Accounts for 15% of the final score (1.5 points out of 10). Students will be evaluated individually, both on their performance during the oral presentation of the selected paperand on the audio-visual material that they prepared to support their group presentation.

5.- Attendance to the oral presentations of research papers

Accounts up to 10% of the final score (1 point out of 10). Both attendance and participation in the scientific discussions of the sessions will be evaluated, following the scale:

Attendance 90-100% = 1 point

Attendance 80-89% = 0,8 points

Attendance 70-79% = 0,7 points

Attendance 60-69% = 0,6 points

Attendance 50-59% = 0,5 points

Attendance 0-49% = 0 points

To be eligible for the retake process, the student should have been previously evaluated in a set of activities equaling at least two thirds of the final score of the course or module. Thus, the student will be graded as "No Avaluable" if the weighthin of all conducted evaluation activities is less than 67% of the final score.

Assessment Activities

Title	Weighting	Hours	ECTS	Learning Outcomes

Attendance to the oral presentations of research papers	10%	0	0	17, 25, 23, 22, 5, 4, 3, 6, 9, 13, 11, 12, 15, 16, 20, 24, 21, 27
Examination of Laboratory classes	15%	1	0.04	17, 1, 25, 2, 10, 23, 22, 5, 4, 3, 6, 8, 7, 9, 13, 11, 12, 14, 18, 15, 16, 20, 24, 19, 21, 27, 26
Final examination of theoretical classes	50%	3	0.12	17, 1, 25, 2, 10, 23, 22, 5, 4, 3, 6, 8, 7, 9, 13, 11, 12, 14, 18, 15, 16, 20, 24, 19, 21, 27, 26
Oral presentations of selected research papers	15%	1	0.04	17, 1, 25, 2, 10, 23, 22, 5, 4, 3, 6, 8, 7, 9, 13, 11, 12, 14, 18, 15, 16, 20, 24, 19, 21, 27
Self-study exercise	10%	1	0.04	17, 1, 25, 2, 10, 23, 22, 5, 4, 3, 6, 8, 7, 9, 13, 11, 12, 14, 18, 15, 16, 20, 24, 19, 21, 27

Bibliography

Bibliografy:

- Transgenic animals. Generation and use. L.M. Houdebine. Harwood Academic Publishers 1997.

- Mouse Genetics and Transgenics. A practical approach. Edited by: I.J. Jackson and C.M. Abbott. Oxford University Press. 2000. (www.oup.co.uk/PAS)

- Gene Targeting. A practical approach. Edited by: A.L. Joyner. Oxford University Press. 2000. (www.oup.co.uk/PAS)

- Manipulating the Mouse Embryo. A laboratory manual. (3rd Edition) Edited by: Andras Nagy et al. Cold Spring Harbor Laboratory Press. 2003.

- Transgenesis Techniques. Principles and Protocols. Edited by: Alan R. Clarke. Humana Press. 2002. (2nd Edition).

- Gene Knock-out Protocols. Edited by: Martin J. Tymms and Ismail Kola. Humana Press. 2001.

- Embryonic Stem Cells. Methods and Protocols. Edited by: Kursad Turksen. Humana Press. 2002.

- Human Molecular Genetics 2. T. Strachan i A.P. Read. John Wiley & Sons, Inc., Publication. 1999.

- Advanced Protocols for Animal Transgenesis. An ISTT Manual. Shirley Pease & Tomas L. Saunders (Editors). Springer. 2011.

- Editando genes: recorta, pega y colorea. Las maravillosas herramientas CRISPR. Lluís Montoliu. Colección el Café Cajal. Next Door Publishers.
2019

Interesting webs:

http://www.transtechsociety.org/

http://www.knockoutmouse.org/

http://www.emmanet.org/