VNIVERSITAT DE VALÈNCIA

Course Guide
43460 Analysis and quantification techniques

COURSE DATA

Data Subject	
Code	43460
Name	Analysis and quantification techniques
Cycle	Master's degree
ECTS Credits	4.5
Academic year	2022 - 2023

Study (s)

Degree	Center	Acad. year	Period
2210 - M.D. in Research in Molecular, Cellular and Genetics Biology	Faculty of Biological Sciences	1	First term
3102 - Biomedicine and Biotechnology	Doctoral School	0	First term

Subject-matter

Degree	Subject-matter	Character
2210 - M.D. in Research in Molecular, Cellular and Genetics Biology	5 - Analysis and quantification techniques	Obligatory
3102 - Biomedicine and Biotechnology	1 - Complementos de Formación	Optional

Coordination

Name	Department
ARTERO ALLEPUZ, RUBEN DARIO	194 - Genetics

SUMMARY

Modern molecular biology and biochemistry aim to unravel the functions of biological systems. Researchers use sophisticated methods that allow imaging and precise data adquisition on cell function, on the expression and structure of genes, and on the interactions between macromolecules. Techniques for gene Analysis and Quantification (TAC) is a multidisciplinary subject, which aims to provide a solid foundation for IBMCG students by covering four methodological blocks: advanced PCR techniques, flow cytometry, in situ nucleic acid detection and alternative splicing, and microscope techniques and imaging analysis. The course is taught jointly by the departments of microbiology, genetics and cell biology.

PREVIOUS KNOWLEDGE

Relationship to other subjects of the same degree

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

Other requirements

OUTCOMES

2210 - M.D. in Research in Molecular, Cellular and Genetics Biology

- Students should apply acquired knowledge to solve problems in unfamiliar contexts within their field of study, including multidisciplinary scenarios.
- Students should be able to integrate knowledge and address the complexity of making informed judgments based on incomplete or limited information, including reflections on the social and ethical responsibilities associated with the application of their knowledge and judgments.
- Students should communicate conclusions and underlying knowledge clearly and unambiguously to both specialized and non-specialized audiences.
- Students should demonstrate self-directed learning skills for continued academic growth.
- To acquire basic skills to develop laboratory work in biomedical research.
- Be able to make quick and effective decisions in professional or research practice.
- Students should possess and understand foundational knowledge that enables original thinking and research in the field.
- Be able to access to information tools in other areas of knowledge and use them properly.
- To be able to assess the need to complete the scientific, historical, language, informatics, literature, ethics, social and human background in general, attending conferences, courses or doing complementary activities, self-assessing the contribution of these activities towards a comprehensive development.
- Capacidad para preparar y gestionar proyectos de investigación en el ámbito de la biología molecular celular y genética.
- Conocer los avances recientes en las técnicas microscópicas y de análisis de imagen, PCR cuantitativa y citometría de flujo comprendiendo su utilidad en distintos campos y las limitaciones de su aplicación.
- Conocer desde un punto de vista práctico los métodos más actuales de marcaje e hibridación de ácidos nucleicos y su aplicación al estudio de la expresión génica in situ.
- Capacidad para interpretar los resultados obtenidos de las técnicas más avanzadas de análisis y cuantificación en biología molecular, celular y genética.

LEARNING OUTCOMES

Be able to design experiments, to understand their applications in various fields and their limitations, and interpret the results obtained through advanced techniques of PCR (including quantitative PCR), flow cytometry, microscopic techniques and image analysis, labeling and in situ hybridization of nucleic acids, and semiquantitative detection of RNA splicing variants.
Be able to coordinate various technologies to solve problems unsolvable with a single technique.
Be able to extrapolate between the areas of technical and conceptual methods developed in other contexts.
Development of analytical skills and the use of logic for understanding complex phenomena. This is a skill that will be acquired in the lab sessions.

DESCRIPTION OF CONTENTS

1. Real time PCR fundamentals.

Chemical basis of the reaction: types of probes. Design and tuning of the reaction: reaction conditions and specificity. Analysis of the dissociation curve. Multiple PCR. Amplification control

2. Quantification by real-time PCR: quantitative PCR (qPCR).

Standard curve as the basis for quantification. Quantification parameters. Efficiency of the reaction. Limit of quantification. Absolute quantification: standard curve method. Relative standard curve. Comparative CT (CT)

3. Applications of qPCR quantification.

RT-qPCR: Expression analysis. NASBA-qPCR. LAMP-qPCR. Quantification of G+C % and DNA/DNA % hybridization (DDH). Quantification of viable cells (v-qPCR). Digital PCR

4. Principles of flow cytometry.

Major systems and components of a cytometer; multiparameter type of information obtained. Fluorophores and fluorescence. Preparation of cells for flow cytometric analysis. Experimental design and data analysis. Advantages and disadvantages of flow cytometry.

5. Main applications of flow cytometry

Measurement of surface parameters: immunophenotyping. Multifluorescent analysis. Analysis of cytoplasmic parameters: intracellular staining. Analysis of DNA ploidy and cell cycle. Study of cell growth. Measurement of apoptosis. Measurement of phagocytic activity and respiratory burst. Measurement of intracellular and secreted cytokines.

6. Cell separation by flow cytometry.

Principles. Characteristics of cells separated by flow cytometry. Purity and yield.

7. General Bases microscopy. Fluorescence Microscopy: multiphoton microscopy and confocal microscopy.

Theoretical and biological applications.

8. Electron microscopy basis. Techniques for subcellular labelling

Theoretical and biological applications. Pre-embedding and post-embedding immunocytochemical staining combined with transmission electron microscopy

9. In situ detection of lacZ reporter and dpp in Drosophila embryos.

We will detect the expression of the lacZ gene in transgenic flies expressing constructs in which a reporter was fused to different cis-regulatory sequences of the rhomboid gene (normal neuroectodermal enhancer or mutated). In parallel, we will detect changes in the expression of the decapentaplegic gene in mutants lacking cabut function or overexpressing this gene. Issues to be discussed will be working under RNase-free conditions, non-radioactive labeling methods, hybridization considerations of nucleic acid detection methods, and signal amplification systems.

10. Quantification of the alternative splicing of the Fhos gene in Drosophila

The aim of this laboratory practice is to determine the effect of CTG expansions on the alternative splicing of the Fhos and shot(short stop) transcripts, the latter serving as control. To this end, we will amplify the relevant fragments of shot and Fhos by RT-PCR, starting from total RNA from adult flies expressing CTG repeats in the muscles and control flies expression no expansions.

WORKLOAD

ACTIVITY	Hours	% To be attended
Theory classes	20,00	100
Laboratory practices	12,00	100
Other activities	8,00	100
Tutorials	3,00	100
Computer classroom practice	2,00	100
TOTAL	**45,00**	

TEACHING METHODOLOGY

The teaching methodology we use is based on learning theory known as constructivism. In short, this theory is based on the idea that learning occurs when students construct new knowledge from reflection on the information supplied to it. Therefore, the role of the teacher in this course will be to promote an intellectually active learning by the student, including the student's reflection on the concepts and principles outlined by the teacher or independently studied.

Lectures and group tutorials: The course is divided into three weekly sessions of one hour. In each session the teacher will present the contents of the agenda items for about 50-55 min. These presentations serve as a theoretical basis for the discussion of items and solving problems and case studies by students to be discussed primarily in tutoring sessions. There will also be guided tours to the research support facilities to show relevant equipment for each technique.

Practical classes: Three real experiments will be performed in the laboratory under the supervision of the teacher. Initially the instructor makes a brief theoretical introduction and presentation of the objectives and methodology, after which each student performs the assigned tasks independently. To help students a manual with theoretical introductions, objectives, methodology and other issues will be available to them before the begining of the exercises. These laboratory exercises are intensive and consist of four sessions of 4 hours each per day.

EVALUATION

The evaluation of the theoretical contents will be made by written tests which, together, constitute 65% of the final grade. This will provide 65% of the final grade.

Practical sessions will be evaluated with the attendance to laboratory sessions and the response to a quiz about them. All this will form the remaining 35% of the grade for the course. Attendance to laboratory sessions and guided tours to central facilities is a requirement to pass the course.

To pass the course it will be necessary to achieve a score of at least 5 points on a total of 10. The final grade is obtained by adding the exam and laboratory assessment, requiring a minimum score of 4 points in the theory exam to pass the course.

REFERENCES

Basic

- 1.- Real-time PCR: an essential guide. 2004. Kirstin Edwards, Julie Logan and Nick Saunders. (Eds). Wymondham (Norfolk). Horizon Bioscience, cop.
  2.- Real-Time PCR: Current Technology and Applications. 2009. Julie Logan, Kirstin Edwards and Nick Saunders (Eds). Applied and Functional Genomics, Health Protection Agency, London. Caister Academic Press.
  3.- Quantitative Real-time PCR in Applied Microbiology. 2012. Martin Filion (Ed). Department of Biology, Université de Moncton, Canada. Caister Academic Press.
  4.- Flow cytometry: principles and applications. 2007. Marion G Macey. Humana Press.
  5.- Practical Flow Cytometry. Howard M. Shapiro. 4ª ed. John Wiley and Sons Inc. Wiley-Liss.
  6.- O´Neil, J.W., Bier, E. (1994). Double-label in situ hybridization using biotin, digoxigenin-tagged RNA probes. Biotechniques 17, 870, con modificaciones.
  7.- Llamusí B, Muñoz-Soriano V, Paricio N, Artero R. (2014). The use of whole-mount in situ hybridization to illustrate gene expression regulation. Biochem Mol Biol Educ. 2014 Jun 30. doi: 10.1002/bmb.20807.