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 / Doctorate
ECTS Credits: 4.5
Academic year: 2026-27

STUDY (S)

Degree	Center	Acad. year	Period
2210 - Master's Degree in Research in Molecular, Cellular and Genetics Biology	Facultat de Ciències Biològiques	1	First quarter

SUBJECT-MATTER

Degree	Subject-matter	Character
2210 - Master's Degree in Research in Molecular, Cellular and Genetics Biology	Analysis and quantification techniques	COMPULSORY

COORDINATION

ARTERO ALLEPUZ RUBEN DARIO

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

COMPETENCES / LEARNING OUTCOMES

2210 - Master's Degree in Research in Molecular, Cellular and Genetics Biology

Be able to access to information tools in other areas of knowledge and use them properly.

Be able to make quick and effective decisions in professional or research practice.

Capacidad para interpretar los resultados obtenidos de las técnicas más avanzadas de análisis y cuantificación en biología molecular, celular y genética.

Capacidad para preparar y gestionar proyectos de investigación en el ámbito de la biología molecular celular y genética.

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.

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.

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.

Students should possess and understand foundational knowledge that enables original thinking and research in the field.

To acquire basic skills to develop laboratory work in biomedical research.

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.

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 (ΔΔCT) 3. Applications of qPCR quantification.RT-qPCR: Expression analysis. NASBA-qPCR. LAMP-qPCR. Quantification of viable cells (v-qPCR). Digital PCR (dPCR): systems, advantages and applications. 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 of 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 a 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 expressing no expansions.

WORKLOAD

PRESENCIAL ACTIVITIES

Activity	Hours
Tutorials	3,00
Theory	19,00
Other activities	8,00
Laboratory	13,00
Computer classroom practice	2,00
Total hours	**45,00**

NON PRESENCIAL ACTIVITIES

Activity	Hours
Attendance at other activities	0,00
Individual or group project	0,00
Independent study and work	40,00
Preparation of lessons	0,00
Preparation for assessment activities	27,50
Resolution of case studies	0,00
Total hours	**67,50**

TEACHING METHODOLOGY

Lectures and Tutorials: The instructor will present the course contents through interactive lectures. These sessions will provide the theoretical foundation for the discussion of scientific articles and for students' resolution of problems and questions. In addition, guided visits to specialized laboratories and central research support facilities will be organized to demonstrate the operation of equipment relevant to each technique.

Practical Sessions: Practical classes will be carried out in the laboratory through the performance of real experiments under the supervision of the instructor. At the beginning of each session, a theoretical and methodological introduction will be provided. Subsequently, students will independently complete the assigned tasks using a laboratory manual that includes the objectives and questions to be addressed, thereby ensuring the effective use of the practical training.

EVALUATION

Assessment

The theoretical component will account for 50% of the final grade and will be assessed through written examinations. Problem-solving activities will represent 30% of the final mark, of which 15% will correspond to the assessment of the practical component and the remaining 15% will derive from the resolution of theory-related problems.

Attendance and participation, which will account for the remaining 20%, will be based on attendance and active participation in the practical sessions. Attendance at practical classes for at least 75% of the scheduled hours is mandatory to pass the course in the first examination period. No minimum mark in the practical component is required to pass the course. Students who attend less than 75% of the practical sessions may still pass the course during the second examination period by completing a questionnaire on the practical content, either together with an examination on the theoretical component if this has also been failed, or separately if the theoretical component has already been passed.

To pass the course, students must obtain a minimum overall score of 5 out of 10 and attend the guided visits. The final grade will be calculated as the sum of the marks obtained in the three assessment components described above. In addition, a minimum score of 4 out of 10 in the theoretical component will be required for the marks from the other components to be combined and for the course to be passed.

REFERENCES

- 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.- Digital PCR. Methods and Protocols. 2018. George Karlin-Neumann and Francisco Bizouarn (Eds). Springer Protocols. Methods in Molecular Biology vol. 1768. Humana Press. 5.- Flow cytometry: principles and applications. 2007. Marion G Macey. Humana Press. 6.- Practical Flow Cytometry. Howard M. Shapiro. 4ª ed. John Wiley and Sons Inc. Wiley-Liss. 7.- O¿Neil, J.W., Bier, E. (1994). Double-label in situ hybridization using biotin, digoxigenin-tagged RNA probes. Biotechniques 17, 870, con modificaciones. 8.- 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.

- En cada tema se proporcionará bibliografía específica, principalmente artículos de investigación o de revisión, que servirá para que los estudiantes puedan profundizar en algunos de los aspectos tratados. Dada su naturaleza, estos artículos se irán actualizando cada año.