Course Unit	Molecular and Metabolic Analysis Techniques			Field of study	Biology and biochemistry				
Master in	Biotechnological Engineering			School	School of Agriculture				
Academic Year	2023/2024	Year of study	1	Level	2-1	ECTS credits	5.0		
Type	Semestral	Semester	1	Code	5010-784-1105-00-23				

Workload (hours)	135	Contact hours	T -	TP -	PL -	TC -	S -	E -	OT -	O -

T - Lectures; TP - Lectures and problem-solving; PL - Problem-solving, project or laboratory; TC - Fieldwork; S - Seminar; E - Placement; OT - Tutorial; O - Other

Name(s) of lecturer(s): Maria Lurdes Antunes Jorge, Rui Miguel Vaz de Abreu

Learning outcomes and competences

At the end of the course unit the learner is expected to be able to:
1. Have knowledge of up to date methods in Molecular Diagnostic, including their theoretical bases
2. Get ability to read, understand and criticize a scientific text
3. Acquire independence in searching data, information, methodologies in order to set up and validate new diagnostic protocols
4. Develop practical specific skills
5. Acquire ability to identify and express relevant information
6. Develop ability to resume and present scientific information
7. Acquire skills at the laboratory level

Prerequisites

Before the course unit the learner is expected to be able to:
Have basic knowledge about genetics and molecular biology

Course contents

DNA fingerprinting and DNA barcoding. PCR and RT-qPCR. Isothermal nucleic acid amplification methods. In silico molecular modeling techniques: molecular docking, QSAR modeling and molecular dynamics simulations. Metabolic engineering methods for the simulation of conditions that enable the production of commercially important metabolites.

Course contents (extended version)

1. DNA “fingerprinting” and DNA “barcoding”
   - Concept. Methodology steps.
   - Core STR loci and human DNA fingerprinting
   - Mitochondrial DNA markers: D-loop hypervariable regions HVR1, HVR2 and HVR3.
   - The role of mitochondrial DNA mutations in several human diseases
   - rDNA and DNA barcoding
2. Polymerase Chain Reaction (PCR)
   - Steps and thermocycling physical and chemical and conditions in endpoint-PCR
   - Primer design guidelines
   - Additives
3. Real Time quantitative PCR (Real-time qPCR or qPCR)
   - Differences between qPCR and standard PCR
   - Real-time qPCR fluorescence production systems: SYBR-green dye and Taqman probes
   - One-step and two-step Reverse Transcription quantitative PCR (RT-qPCR)
   - Reverse Transcriptases
   - Controls for qRT-PCR (NTC; NRT)
   - Melting-curve analyses
   - Interpretation of results
   - Methods of quantification
4. Techniques based in isothermal nucleic acid amplification: LAMP
   - Uses and advantages
5. Methods of analysis, modeling and simulation of 3D structures of different biomolecules
   - Tools for visualization and manipulation of three-dimensional structures of biomolecules
   - Application in the study of the molecular mechanism of action of molecules with bioactive properties
6. In silico techniques for predicting the potential of molecules as inhibitors of target proteins
   - Protein-ligand and protein-protein molecular docking
   - Molecular dynamics simulations and QSAR models
   - Integration of these techniques in the process of developing new drugs
7. Strategies for simulating production conditions of metabolites with commercial interest
   - In silico tools for the study and manipulation of the metabolism of different microorganisms
   - Analysis of flows of central metabolism: networks and metabolic pathways, concept of metabolic flows
   - Simulation of genetic modifications for the production of metabolites of commercial interest

Recommended reading

1. Jordan, D & Mills, D. (2021). Past, Present, and Future of DNA Typing for Analyzing Human and Non-Human Forensic Samples. Front. Ecol. Evol. 9. https: //doi. org/10. 3389/fevo. 2021. 646130
2. Aragona, M. (2022). New-Generation Sequencing Technology in Diagnosis of Fungal Plant Pathogens: A Dream Comes True? J. Fungi, 8, 737. https: //doi. org/10. 3390/jof8070737
3. Taylor, S. & Mrkusich, E. (2014). The State of RT-qPCR: Firsthand Observations of Implementation of MIQE. J. Mol. Microbiol. Biotechnol. 24: 46–52. https: //www. karger. com/Article/FullText/356189
4. Temitope, Isaac et al (2021) Molecular modeling in drug discovery. Informatics in Medicine Unlocked 29 (2022)
5. Tafur, Rangel et al (2021) In silico Design for Systems-Based Metabolic Engineering for the Bioconversion of Valuable Compounds From Industrial By-Products. Front. Genet. 12: 633073

Teaching and learning methods

Theoretical classes - Expositive methodology, using audiovisual means. Study materials available through e-learning resources. Practical classes - execution of laboratory protocols

Assessment methods

1. Alternative 1 - (Regular, Student Worker) (Final)
   - Intermediate Written Test - 50% (Practical and theoretical written test - items 1 to 4 of the program; minimum grade is 8 out of 20)
   - Final Written Exam - 30% (Practical and theoretical written test - items 5 to 7 of the program; minimum grade is 8 out of 20)
   - Reports and Guides - 20% (Reports about projects related to molecular modeling and in silico metabolic engineering)
2. Alternative 2 - (Regular, Student Worker) (Supplementary, Special)
   - Final Written Exam - 100% (Final written exam; minimum grade is 9. 5 out of 20)

Language of instruction

English

Electronic validation

Maria Lurdes Antunes Jorge, Rui Miguel Vaz de Abreu	Altino Branco Choupina	Rui Miguel Vaz de Abreu	Paula Cristina Azevedo Rodrigues
24-01-2024	24-01-2024	24-01-2024	25-01-2024