

| 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  |
| Туре             | Semestral                                   | Semester      | 1                             | Code           | 5010-784-1105-00-23                           |   |
| Workload (hours) | 135   | Contact hours | T - Lectures; TP - Lectures a | - PL - T       | C - S -<br>solving, project or laboratory; TC | E - OT - O -<br>- 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
- Acquire independence in searching data, information, methodologies in order to set up and validate new diagnostic protocols.
   Develop practical specific skills
   Acquire ability to identify and express relevant information
   Develop ability to resume and present scientific information

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- 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)

- 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

   Polymerase Chain Reaction (PCR)

   Steps and thermocycling physical and chemical and conditions in endpoint-PCR
   Primer design guidelines
   Additives
  - Additives
- Additives
   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: LAMF
- Uses and advantages
- Uses and advantages
  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

  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. concent of metabolic flow

  - 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

- 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
   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.
- LAYIOL, S. & WILKUSICH, E. (2014). THE State of RT-9PCR: Firstnand Observations of Implementation of MIQE. J. Mol. Microbiol. Biotechnol. 24: 46–52. https://www.karger.com/Article/FullText/356189
   Temitope, Isaac et al (2021) Molecular modeling in drug discovery. Informatics in Medicine Unlocked 29 (2022)
   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

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# Assessment methods

- 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)

   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<br>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                       |