

Course Unit	Molecular Diagnosis Methods		Field of study	Biology and biochemistry	
Master in	Biotechnological Engineering		School	School of Agriculture	
Academic Year	2021/2022	Year of study	1	Level	2-1
Type	Semestral	Semester	2	ECTS credits	5.0
Code	5010-509-1203-00-21				
Workload (hours)	135	Contact hours	T 25	TP -	PL 25
			TC -	S -	E -
			OT 4	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

### 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 Polymorphisms and DNA molecular markers. Polymerase Chain Reaction (PCR). Mutation detection methods based in isothermal amplification. Mutation detection methods based in nucleic acid hybridization: FISH and microarrays. Applications in medicine and pharmacogenomics: diagnosis, prognosis and therapy. Sequencing techniques: Sanger (ddNTPs), Next Generation System: pyrosequencing, sequencing by synthesis, by ligation and Nanopore sequencing. RNA interference (RNAi) and eucariotic gene regulation. CRISPR-Cas9 genome editing.

### Course contents (extended version)

1. DNA Polymorphisms and DNA molecular markers
  - DNA Polymorphisms: VNTRs, STRs and RFLPs. STRs and DNA fingerprinting. SNPs.
  - Other nuclear DNA markers (ESTs, SSCP, DGGE, TGGE, DHPLC and SCAR)
  - Mitochondrial DNA markers: D-loop hypervariable regions HVR1 and HVR2.
  - The involvement of mitochondrial DNA in several human diseases
  - rDNA and DNA barcoding
2. Polymerase Chain Reaction (PCR)
  - Steps and thermocycling physical conditions in endpoint-PCR
  - One-step and two-step Quantitative Real-Time PCR (qRT-PCR). Real-time PCR components and phases
  - Controls for qRT-PCR (NTC; NRT)
  - Real-time systems: SYBR-green, Taqman probes, Scorpions. . .
  - Melting-curve analyses
  - Mutation detection methods based in other PCR variants: ARMs, OLA, ASO
3. Mutation detection methods based in isothermal amplification
  - LAMP, SDA, TMA, NASBA
  - Advantages of isothermal amplification
4. Mutation detection methods based in nucleic acid hybridization
  - Southern and Northern blot
  - FISH
  - Microarrays: gene expression analysis and genetic variation analysis. aCGH
  - Applications in medicine and pharmacogenetics: diagnosis, prognosis and therapy
5. Sequencing techniques: Sanger, NGS: pyrosequencing, by synthesis, by ligation and by nanopores
6. RNA interference (RNAi) and eucariotic gene regulation.
7. CRISPR-Cas9 genome editing

### Recommended reading

1. Coleman WB, Tsongalis GJ (2005) Molecular Diagnostics for the Clinical Laboratorian, 2nd Ed, Humana Press
2. Wilson, K. & Walker, J. (2010). Principles and Techniques of Biochemistry and Molecular Biology. 7th edition. Cambridge University Press. (ISBN 9780521516358)
3. O'Connor, C. (2008). Fluorescence in situ hybridization (FISH). Nature Education 1(1): 171.
4. Wolska, K. & Szveda, P. (2012). Genotyping Techniques for Determining the Diversity of Microorganisms. ISBN: 978-953-51-0064-5, InTech.
5. Nature video (2017). CRISPR: Gene editing and beyond. <https://www.youtube.com/watch?v=4YKFw2KZA5o>

### 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 Oral Test - 15% (Practical laboratory exam)
  - Intermediate Written Test - 25% (Written practical test (minimum grade is eight out of twenty)
  - Intermediate Written Test - 20% (Partial theoretical assessment throughout the semester)
  - Final Written Exam - 40% (Final written test about all the theoretical subject content (minimum grade is eight out of twenty)
2. Alternative 2 - (Regular, Student Worker) (Supplementary, Special)
  - Final Written Exam - 100% (Final Exam. To approve student will have to have at least nine dot five of twenty)

## Language of instruction

English

## Electronic validation

Maria Lurdes Antunes Jorge	Altino Branco Choupina	Paula Cristina Azevedo Rodrigues	Maria José Miranda Arabolaza
06-12-2021	06-12-2021	06-12-2021	06-12-2021