General Information	BACELOR DEGREE IN BIOTECHONOLOGIES
Title of the subject	Cellular engineering and laboratory of cellular technologies
Degree Course (class)	Industrial and Agro-Food Biotechnology (L-2)
ECTS credits	6
Compulsory attendance	yes
Language	Italian with slides in English
Academic year	2020/2021

Subject Teacher		
Name and Surname	Rosa Angela Cardone	
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Place and time of reception	Nuovo Palazzo dei Dipartimenti Biologici, IV Piano. Stanza N. 47. Campus dell'Università degli Studi di Bari "Aldo Moro". Via Orabona, 4, Bari (BA). Monday 10.00-12.00	
ECTS credits details	Discipline sector (SSD)	Area
	BIO/09	---

Study plan schedule	Year of study plan	Semester
	3°	2°

Time management	Lessons	Laboratory	Exercises	Total
CFU	3	3		6
Total hours	75	75		150
In-class study hours	24	36		60
Out-of-class study hours	51	39		90

Syllabus	
Prerequisites / Requirements	
Elements of Biochemistry, Molecular Biology, Physiology	
Expected learning outcomes (according to Dublin descriptors)	
Knowledge and understanding	Students will demonstrate to have acquired notions related to cell manipulation (i.e. bacteria, yeasts, mammalian stem and differentiated cells) to study the structure and function of proteins of interest, both for research and industrial purposes.
Applying knowledge	Students will demonstrate to understand and address specific issues related to the above points, with the ultimate goal of independently designing the entire process of manipulating cells and animals as a whole.
Making informed judgments and choices	Students should demonstrate the acquisition of all the necessary tools for the evaluation of both research and industrial activity cases. They should be able to independently design each experimental phase, from the design of specific genes to their manipulation strategies to the characterization and evaluation of the functionality

	of the expressed protein.
Communicating knowledge	Students should demonstrate to have acquired the correct technical terminology necessary for both communication and the construction of a network of collaborations.
Capacities to continue learning	Students must be able to analyze and understand texts and to deepen problems through specific bibliography
Study Program	
Content	**Introduction to cellular engineering** Definitions. Targets. Definition of recombinant DNA and recombinant proteins **Recombinant proteins** The expression systems in E.Coli, in yeast cells and in mammalian cells. Production and purification of fusion proteins. The biotechnological applications of cloning: the production of pharmaceutical ingredients with recombinant DNA technology. The production of recombinant insulin. The synthesis of human growth hormones in E. coli. The production of recombinant factor VIII. The synthesis of commercial products by recombinant microorganisms. **Cell cultures** Primary and secondary crops. Two-dimensional (2D) crops. Three-dimensional (3D) scaffolds-free cultures and natural and synthetic scaffolds. The organoids. The transfection of eukaryotic cells using physical and chemical methods for the expression of recombinant proteins Electroporation, microinjection, the technique of Ca2 + / phosphate, DEAE-dextran, liposomes of viral vectors. **Gene silencing** Mechanism of RNA interference (RNAi). Enzyme complexes involved in the process: DICER, RISC multi-protein compress (RNA - Induced Silencing Complex). Length and choice of position of siRNAs. Expression vectors for siRNA production: strategies, shRNA, plasmid and viral vectors. RNAi in vitro and in vivo: experimental strategies. **I*n vitro* analysis of cellular recombinant proteins (qualitative and quantitative assays)** Protein extraction and purification, protein analysis by SDS-PAGE and Western Blotting, immunoprecipitation, kinase activity assays. Protein-protein interaction studies: the "pull-down". Cell viability, apoptosis, cell migration and invasion assays. **In vivo analysis of cellular recombinant proteins (localization, subcellular dynamics and functions)** Conventional and confocal optical microscopy. Immunofluorescence and immunocytochemical techniques. The use of fusion proteins for the analysis of protein functions: the "Fluorescence Resonance Energy Transfer" (FRET).

	Stem Cells Definition of regeneration and repair. Definition and classification in totipotent, pluripotent, multipotent Types of stem cells: embryonic, fetal, cord and placental stem cells, adult stem cells. Derivation and culture of embryonic stem cells. Reprogramming of differentiated cells into induced pluripotent stem cells. **Laboratories (to be defined according to the research activities in progress)** Maintenance of stabilized human (2D) cell cultures. Set up of 3D cell cultures Transfection of recombinant proteins in eukaryotic cells by cationic lipids. Protein expression characterization of recombinant proteins Functional characterization of recombinant proteins: growth, cytotoxicity and motility assays Characterization of the *in situ* expression and interaction of recombinant proteins by Proximity Ligation Assay Analysis of recombinant proteins by imaging techniques (Epifluorescence and / or Confocal and / or FRET)
Bibliography and textbooks	T.A. Brown. "Biotecnologie Molecolari" –Zanichelli. Handouts and PPT slides provided by the teacher.
Notes to textbooks	All information concerning the texts and scientific articles included in the program is available from the teacher or online.
Teaching methods	- Frontal lessons with PPT support - Frontal lessons with video projection and comments - Single seat laboratory exercises - Virtual laboratory activities with PPT and commented videos
Assessment methods (oral, written, ongoing assessment)	Oral examination
Evaluation criteria (describe criteria for each of the above expected outcomes)	To pass the exam it is necessary that the student demonstrates that he/she has achieved the expected results (described above) at a level that allows him/her to easily discuss, having good ability to integrate the various topics covered during the course, as these are closely connected between them.
Further information	