<table>
<tr><td colspan="2">NAME OF THE COURSE</td><td colspan="5">Molecular Biotechnology</td></tr>
<tr><td>Code</td><td>PMB707</td><td>Year of study</td><td colspan="4">1.</td></tr>
<tr><td>Course teacher</td><td>Jasna Puizina, PhD, Professor</td><td>Credits (ECTS)</td><td colspan="4">7,0</td></tr>
<tr><td rowspan="2">Associate teachers</td><td rowspan="2">Željana Fredotović, PhD, Assistant Professor; Ivica Šamanić, PhD, Assistant Professor</td><td rowspan="2">Type of instruction (number of hours)</td><td>L</td><td>S</td><td>E</td><td>F</td></tr>
<tr><td>30</td><td>15</td><td>45</td><td></td></tr>
<tr><td>Status of the course</td><td>Mandatory</td><td>Percentage of application of e-learning</td><td colspan="4">10%</td></tr>
<tr><td colspan="6">COURSE DESCRIPTION</td></tr>
<tr><td>Course objectives</td><td colspan="5">Lectures: students will be introduced to the main techniques and methods of molecular biotechnology and some of its most common applications.
Seminars: students will, through solving problem and numerical tasks, improve their understanding of the accepted knowledge and concepts and will orally explain the selected research article individually or in the group. They will discuss some ethical dilemmas in molecular biotechnology.
Practical work: students will independently, in pairs or in groups, conduct practical laboratory experiments and at least one smaller research project.</td></tr>
<tr><td>Course enrolment requirements and entry competences required for the course</td><td colspan="5">Cell biology, Genetics, Molecular biology, Biochemistry</td></tr>
<tr><td>Learning outcomes expected at the level of the course (4 to 10 learning outcomes)</td><td colspan="5">After completion of the course, students will be able to:
• Create recombinant DNA using key techniques and methods of recombinant DNA technology (genetic engineering).
• Analyze genetic expression by different techniques
• Analyze interactions between proteins, proteins and nucleic acids
• Design and implement a simpler CRISPR / Cas9 gene change experiment
• Understand how recombinant DNA methodology can be useful in understanding the functions of individual genes
• Explain how manipulation of nucleic acids and proteins can create new properties in transgenic organisms
• Argue the risks and benefits of using recombinant DNA technology and genetically modified organisms.
• Use scientific literature and on-line databases.
• Use standard and specialized laboratory equipment</td></tr>
<tr><td>Course content broken down in detail by weekly class schedule (syllabus)</td><td colspan="5">Lectures (30 hrs):
1. Introduction, development of molecular biotechnology, recombinant DNA technology, commercialization
2. Restriction enzymes: nomenclature, DNA recognition and cutting methods, restriction mapping. Other enzymes in molecular cloning: DNA and RNA polymerase, DNase, RNase, ligase, kinase, phosphorylase, topoisomerase, reverse transcriptase.</td></tr>
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	3. DNA-cloning vectors: plasmids, viral Vectors, cosmids, bacterial artificial chromosome, yeast artificial chromosome, human artificial chromosome, shuttle vectors 4. Various DNA cloning methods: cloning using restriction enzymes, TA and TOPO-TA cloning, Gatewey cloning. 5. Introduction of recombinant DNA into host cells: transformation, transfection, electroporation, transduction. Selection methods of successfully transformed cells. 6. Gene libraries, creation, amplification. Recombinant DNA detection methods among clones or gene libraries (genetic, hybridizing, immunological, etc.) 7. Cloning eukaryotic genes, obtaining cDNA from mRNA. 8. Gene expression analysis: Northern-blot, Reverse transcription (RT-PCR) and Real-Time PCR or qPCR. 9. Expression of recombinant DNA into prokaryotes and eukaryotes. Combinations of the vector and the corresponding host (bacteria, yeasts, animal and plant cells). Regulated expression - inducible / repressive. Use of reporter genes and fusion genes. (2 hrs) 10. Protein isolation (affinity chromatography), protein detection (SDS-PAGE, Western hybridization, ELISA, localization of antibodies in cells and tissues. 10. Protein isolation (affinity chromatography), protein detection (SDS-PAGE, Western hybridization, ELISA, localization of antibodies in cells and tissues. 11. Interaction analysis between proteins. GST pull-down, immunoprecipitation. Yeast two-hybrid system, Tandem TAP purification. 12. Immune system, antibodies, and their most commonly used applications in biosciences and medicine. (2 hrs) 13. Molecular Diagnosis and Protein Therapeutics, Nucleic Acids as Therapists, Vaccines. (2 hrs) 14. Recombinant microorganisms. Application in industry and the environment. (2 hrs) 15. Production of large quantities of protein from recombinant microorganisms. Bacterial growth, fermentation, bioreactors. (2 hrs) 16. Transgenic animals – methods of creating: DNA microinjection, retroviral vectors, embryonic stem cells, conditional mutations (Cre-loxP system), Crispr / Cas9 and genomic engineering, RNA interference. (2 hrs) 17. Transgenic animals - application: transgenic mouse models of the disease, test systems, gene expression control and cell death. Application in pharmacy, agriculture, medicine. (2 hrs) 18. Gene therapy: structure of viruses that serve as gene vectors: adenoviruses, retroviruses and herpes viruses, viral vectors in tumor gene therapy and vaccination, non-viral gene uptake procedures, gene therapy for cancer, monogenic diseases and for regenerative medicine. (2 hours) 19. Transgenic Plants - Methods: Ti plasmid, gene gun, chloroplast engineering, gene reporter, gene expression manipulation, transgenic plants marker-free. (2 hrs) 20. Transgenic plants - application: resistance to herbicides, viruses, insects, fungi, nutritional composition modifications, taste and appearance, edible vaccines, yield and yield. 21. Molecular biotechnology and society.

	Seminars (15 hrs) 1. Solving problem and numerical tasks. (5 hours) 2. Present selected scientific articles individually, in pairs or in a group. (5 hours) 3. Oxford debate about several ethical dilemmas chosen. (2 hours) 4. Presentation of the results of the mini-projects performed on the practicals of this course. **Laboratory exercises (45 hours):** Practical exercises are planned as 4 smaller student project assignments. 1. Identification of an unknown herbal and animal sample by genetic cloning (in vitro and in vivo). DNA isolation, amplifying the barr-coding sequence DNA: ITS region in the plant genome and the cytochrome oxidase I (COI) gene in the animal genome. Gel electrophoresis, fragment isolation from gel agarose. TOPO-TA cloning of the obtained PCR product (restriction enzymes and vectors), E. coli transformation, white-blue selection, overnight culture, plasmid isolation. Sequence of obtained clones, bioinformatics sequence analysis, identification of sequences using similar / identical to Genbank (online blast-quest). Interpretation of results. (10 hours) 2. Expression of recombinant protein. Working with expression vectors, selected genes and host cells. Production of a recombinant protein. Protein isolation. Detection and visualization of proteins. (10 hours) 3. Gene expression analysis. Students will select the gene as well as the cell type (organism). Primers design. Treatment of experimental material and controls. Isolation of total RNA, denaturating electrophoresis, RNA quantification, cDNA synthesis, real-time PCR quantification. (12 hours) 4. Using CRISPR / Cas9 technology. Students will select a gen and design a sgRNA for its inactivation or modification. Preparation of a recombinant vector, host transformation, interpretation of results (13 hours)
Format of instruction	☒ lectures ☒ seminars and workshops ☒ exercises ☐ *on line* in entirety ☒ partial e-learning ☐ field work ☒ independent assignments ☒ multimedia ☒ laboratory ☐ work with mentor ☐ (other)
Student responsibilities	Students are obliged to be present in the amount of at least 70% of scheduled lectures. They are also obliged to give a seminar, perform all laboratory exercises, and to write a written report.

Screening student work *(name the proportion of ECTS credits for each activity so that the total number of ECTS credits is equal to*						
	Class attendance	1	Research		Practical training	2
	Experimental work	1	Report		Other)	
	Essay		Seminar essay	1	(Other)	
	Tests		Oral exam		(Other)	
	Written exam	2	Project		(Other)	

the ECTS value of the course)						

Grading and evaluating student work in class and at the final exam	Active participation of students in the classroom is scored as follows: inadequate (1) student does not participate actively in the classes; a sufficient (2) student actively participates in teaching only after the question is asked, a good (3) student occasionally actively participates in the lessons but hardly makes independent conclusions; very good (4) student often actively participates in teaching and often makes independent conclusions; an excellent (5) student almost always actively participates in teaching, critically reflects and independently brings conclusions. A written exam is passed if the student achieves at least 55% of the total number of points. Scoring: <55% of students did not satisfy; 55-67% sufficient (2); 68-78% good (3); 79-89% very good (4); 90-100% excellent (5). The final grade represents a combination of individual grades 1) active participation in teaching, 2) practical work, 3) seminar work, 4) written exam.		
Required literature (available in the library and via other media)	**Title**	**Number of copies in the library**	**Availability via other media**
	B.R., Glick, C.L., Patten: Molecular biotechnology – Principles and Applications of Recombinant DNA. 2017. American Society of Microbiology.	1	
	Internal script with instructions and protocols for practical exercises	-	yes
	Power point presentations in PDF format	-	yes
	Selected original and review scientific articles	-	yes
Optional literature (at the time of submission of study programme proposal)	• Renneberg, Biotechnology for Beginners, Academic Press, 2008. • Clark, Pazdernik, Biotechnology, Academic Press, 2012. • Thieman, Palladino, Introduction to Biotechnology, Pearson, 2014.		
Quality assurance methods that ensure the acquisition of exit competences	Quality and performance monitoring will be carried out at three levels: (1) university, (2) faculty, through the Quality Control Committee, (3) teaching level.		
Other (as the proposer wishes to add)			