

### UČNI NAČRT PREDMETA / COURSE SYLLABUS

Predmet:	Izbrane metode v biokemiji in molekularni biologiji
Course title:	Selected Methods in Biochemistry and Molecular Biology

Študijski program in stopnja Study programme and level	Študijska smer Study field	Letnik Academic year	Semester Semester
Univerzitetni študijski program: Ekologija, 1. stopnja		2. ali 3.	3. ali 4. ali 5. ali 6.
Undergraduate university programme: Ecology with Nature Conservation, 1st level		2nd or 3rd	3rd or 4th or 5th or 6th

Vrsta predmeta / Course type	izbirni/Elective
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Univerzitetna koda predmeta / University course code:	
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Predavanja Lectures	Seminar Seminar	Sem. vaje Tutorial	Lab. vaje Laboratory work	Teren. vaje Field work	Samost. delo Individ. work	ECTS
15			30		135	6

Nosilec predmeta / Lecturer:	Janja TRČEK
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Jeziki / Languages:	Predavanja / Lectures: Vaje / Tutorial:	Slovenski /Slovenian Slovenski /Slovenian
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Pogoji za vključitev v delo oz. za opravljanje  
študijskih obveznosti:

Jih ni	No
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**Vsebina:**

Začetna predavanja bodo namenjena ponovitvi strukture genov in genomov. Sledile bodo predstavitev molekularno-bioloških tehnik za analizo proteinov, kromosomov in nukleinskih kislin. Za tem bodo predstavljene tehnikе vzorčenja različnih vrst celic, metode izolacije, čiščenja in kvantifikacije nukleinskih kislin ter separacija DNA in RNA z elektroforezo na agaroznih in poliakrilamidnih gelih ter izolacija nukleinskih kislin iz gelov. Predstavljene bodo tudi metode encimske modifikacije DNA, kot so rezanje z restriktijskimi encimi, lepljenje, fosforilacija in označevanje nukleinskih kislin. Razloženi bodo principi kloniranja, priprave DNA-knjžnic, izolacije pozitivnih klonov, izražanja rekombinantnih proteinov v različnih ekspresijskih sistemih kakor tudi uporaba različnih vektorjev za kloniranje in ekspresijo. Podane bodo metode hibridizacije nukleinskih kislin, pomembne za pregledovanje DNA-knjžnic, za metodi prenosa RNA po Northernu in DNA po Southernu in za kvantitativno ocenjevanje sorodnosti biotskih vrst na osnovi genomske DNA. Razloženi bodo tudi principi in možnosti uporabe tehnike verižne reakcije s polimerazo (PCR) in principi sekvenciranja DNA. Nazadnje bodo podane osnove analize nukleinskih kislin s pomočjo različnih bioinformatskih orodij (npr. BLAST in CLUSTAL) ter različni pristopi in bioinformatska orodja za analizo filogenetskih odnosov med organizmi.

**Content (Syllabus outline):**

The introductory lectures will be dedicated to a review on genes and genome structures. Then the molecular-biological techniques on analyses of proteins, chromosomes and nucleic acids will be presented. Sampling techniques for different cell types will be presented and the methods for nucleic acids isolation, purification, separation and quantification using agarose and polyacrylamide electrophoresis will be given. Recovery of DNA and RNA fragments from gels will also be presented. Methods for enzymatic manipulation of DNA such as digestion of DNA with restriction enzymes, ligation, phosphorylation and nucleic acids labeling will be presented. The principles of cloning DNA, construction of DNA libraries, isolating positive clones, expression of recombinant proteins in different expression systems, the use of different cloning and expression vectors will be explained. Nucleic acid hybridization methods, important for screening DNA-libraries as well as for Northern and Southern blot methods and for quantitative assessment of relatedness of biotic species on the basis of genomic DNA will be given. The principles and possible applications of polymerase chain reaction (PCR) and the principles of different sequencing technology will also be explained. Finally, the fundamentals of nucleic acid analyses using different bioinformatics tools (such as BLAST and CLUSTAL) will be given and different approaches and bioinformatics tools for the analyses of phylogenetic relationships from molecular data.

**Temeljni literatura in viri / Readings:**

- Berg J.M., Tymoczko J.L., Gatto G.J., Stryer L. 2015. Biochemistry. 8. izdaja, WH Freeman, 1056 str.
- Krebs J.E., Goldstein E.S., Kilpatrick S.T. 2017. Lewin's Genes XII. 12. izdaja, Jones & Barlett Publisher, 838 str.
- Sambrook J., Russell, D.W. 2001. Molecular Cloning – a Laboratory Manual. 3. izdaja, Cold Spring Harbor Laboratory, 999 str.

**Cilji in kompetence:**

Namen predmeta je podati osnovne informacije kakor tudi spremljajoč teorijo, oziroma principe na katerih temeljijo številne molekularno-biološke tehnike, med katerimi so mnoge uporabljeni tudi v klasičnih bioloških disciplinah. Velik poudarek je na praktičnemu laboratorijskemu delu. Študenti naj bi obvladali številne tehnike, kot so:

- Izolacija RNA in DNA iz različnih vrst celic
- Čiščenje nukleinskih kislin
- Kvantifikacija nukleinskih kislin
- Separacija RNA in DNA na agaroznih gelih
- Separacija proteinov na poliakrilamidnih gelih
- Encimske modifikacije
- Kloniranje DNA in priprava DNA knjižnic
- Transformacija DNA v različne celične sisteme
- Hibridizacija RNA in DNA
- Prečesavanje pozitivnih klonov
- Northern blot
- Southern blot
- Polimerazna verižna reakcija (PCR)
- Sekvenciranje DNA

**Objectives and competences:**

The aim of the subject is to give background information as well as underlying theory of the main molecular biology methods currently used, many of them applied also in systematics, evolution and ecology. A great emphasis should be done on practical laboratory work. Students should be able to use many techniques, including:

- RNA and DNA isolation from various cell types
- Nucleic acid purification
- Nucleic acid quantification
- Separation of RNA and DNA on agarose gels
- Separation of proteins on polyacrylamide gels
- Enzyme modifications
- DNA cloning and DNA library construction
- Transformation of DNA in different cell systems
- Hybridization of RNA and DNA
- Screening of positive clones
- Northern blot
- Southern blot
- Polymerase chain reaction (PCR)
- DNA sequencing

**Predvideni študijski rezultati:****Znanje in razumevanje:**

- Študenti dobijo podroben vpogled v strukturo genov in genomov ter se zananijo z možnostmi in načini uporabe molekularno-bioloških tehnik pri študiju biodiverzitete in filogenije.
- Poleg poznавanja principov številnih molekularno-bioloških metod in sposobnosti kritičnega ovrednotenja njihove uporabe v praksi dobro obvladajo laboratorijsko delo z različnimi aparaturami ter ravnanje z zdravju škodljivimi kemikalijami.

**Intended learning outcomes:****Knowledge and Understanding:**

- Students get a detailed insight in gene and genomes structures, and about possibilities and ways of applications of molecular-biology techniques in biodiversity and phylogenetic.
- Besides the knowledge of principles of numerous molecular biology methods and the capacity of critical evaluations of their applications in practice, they are familiar with various laboratory instruments as well with handling with toxic materials.

Prenesljive/ključne spretnosti in drugi atributi:	Transferable/Key Skills and other attributes:
<ul style="list-style-type: none"> <li>• Obvladovanje številnih sodobnih metod v molekularni biologiji</li> <li>• Poznavanje osnovnih pristopov in sposobnost uporabe bioinformatskih orodij pri analizi nukleinskih kislin kakor tudi filogenetskih povezav s pomočjo molekularno bioloških podatkov</li> </ul>	<ul style="list-style-type: none"> <li>• Capacity of understanding and handling numerous currently used methods in molecular biology.</li> <li>• Knowledge of basic approaches and the ability to use current software applications for analyses of nucleic acids as well as phylogenetic relationships using molecular biology data.</li> </ul>

Metode poučevanja in učenja:	Learning and teaching methods:			
<ul style="list-style-type: none"> <li>• Predavanja</li> <li>• Laboratorijske vaje</li> <li>• Individualno delo s študenti</li> </ul>	<ul style="list-style-type: none"> <li>• Lectures</li> <li>• Laboratory excercises</li> <li>• Individual works with students</li> </ul>			
Načini ocenjevanja:	Delež (v %) / Weight (in %)	Assessment:		
<ul style="list-style-type: none"> <li>• Kolokvij</li> <li>• Pisni izpit</li> </ul>	<table border="1"> <tr> <td>50</td> <td>50</td> </tr> </table>	50	50	<ul style="list-style-type: none"> <li>• Practical examination</li> <li>• Written examination</li> </ul>
50	50			

Reference nosilca / Lecturer's references:
<ol style="list-style-type: none"> <li>1. Lee C., Franke K.B., Kamal S.M., Kim H., Lünsdorf H., Jäger J., Nimtz M., <b>Trček J.</b>, Jänsch L., Bukau B., Mogk A., Römling U. 2018. Stand-alone ClpG disaggregase confers superior heat tolerance to bacteria. Proc. Natl. Acad. Sci. USA 115 (2):E273-E282.</li> <li>2. Škraban J., Kyrpides N.C., Shapiro N., Whitmann W.B., <b>Trček J.</b> 2018. Draft genome sequence of <i>Chryseobacterium limigenitum</i> SUR2<sup>T</sup> (LMG 28734<sup>T</sup>) isolated from dehydrated sludge. Braz. J. Microbiol. 49 (1), 5-6.</li> <li>3. <b>Trček J.</b>, Mahnič A., Rupnik M. 2016. Diversity of the microbiota involved in wine and organic apple cider submerged vinegar production as revealed by DHPLC analysis and next-generation sequencing. Int. J. Food Microbiol. 223, 57-62.</li> <li>4. <b>Trček J.</b>, Lipoglavšek L., Avguštin G. 2016. 16S rRNA <i>in situ</i> hybridisation followed by flow cytometry for rapid identification of acetic acid bacteria involved in submerged industrial vinegar production. Food Technol. Biotechnol. 54 (1), 108-112.</li> <li>5. <b>Trček, J.</b>, and K. Matsushita. 2013. A unique enzyme of acetic acid bacteria, PQQ-dependent alcohol dehydrogenase is also present in <i>Frateuria aurantia</i>. Appl. Microbiol. Biotechol., 97, 16, 7369-7376.</li> </ol>