| UČNI NAČRT PREDMETA / COURSE SYLLABUS | |
|---|---|
| **Predmet:** | MOLEKULSKO KLONIRANJE |
| **Course Title:** | MOLECULAR CLONING |

| Študijski program in stopnja<br>Study Programme and Level | Študijska smer<br>Study Field | Letnik<br>Academic Year | Semester<br>Semester |
|---|---|---|---|
| **UŠP Biokemija, 1. stopnja** | **/** | **3.** | **5.** |
| **USP Biochemistry, 1st Cycle** | **/** | **3rd** | **5th** |

**Vrsta predmeta / Course Type:** obvezni / Mandatory

**Univerzitetna koda predmeta / University Course Code:** BK131

| Predavanja<br>Lectures | Seminar<br>Seminar | Vaje<br>Tutorial | Klinične vaje<br>Work | Druge oblike študija | Samost. delo<br>Individual Work | ECTS |
|---|---|---|---|---|---|---|
| **30** | **5** | **40 LV** | **/** | **/** | **75** | **5** |

**Nosilec predmeta / Lecturer:** prof. dr. Marko Dolinar / Dr. Marko Dolinar, Full Professor

**Jeziki / Languages:**

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| **Predavanja / Lectures:** | **slovenski / Slovenian** |
| **Vaje / Tutorial:** | **slovenski / Slovenian** |

| Pogoji za vključitev v delo oz. za opravljanje študijskih obveznosti: | Prerequisites: |
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| Študent oz. kandidat mora imeti predmet opredeljen kot študijsko obveznost. | The course has to be assigned to the student. |

| Vsebina: | Content (Syllabus outline): |
|---|---|
| 1. Raziskovanje DNA in molekularna biotehnologija: zgodovina in prihodnost.<br>2. Laboratorijski organizmi v DNA-tehnologiji.<br>3. Encimi pri delu z DNA.<br>4. Elektroforezne metode.<br>5. Vektorske molekule. Reporterski geni.<br>6. Sinteza DNA *in vitro*. Sinteza cDNA. Priprava DNA-knjižnic. Transformiranje celic.<br>7. PCR in izvedene tehnike.<br>8. Hibridizacija, sonde, načini označevanja DNA. Presejanje knjižnic.<br>9. Določanje nukleotidnega zaporedja DNA. | 1. Exploring DNA and molecular biotechnology – past and future.<br>2. Laboratory organisms in DNA technology.<br>3. DNA-modifying enzymes.<br>4. Electrophoretic methods.<br>5. Vectors and reporters.<br>6. DNA synthesis in vitro. cDNA synthesis. Preparation of DNA libraries. Cell transformation.<br>7. PCR and deducted techniques.<br>8. Hybridization, probes and approaches for DNA labelling. Screening of DNA libraries.<br>9. DNA sequencing.<br>10. Expression systems. In vitro |

10. Ekspresijski sistemi. Transkripcija/translacija *in vitro*.
11. Izražanje v prokariontih: vektorji, fuzije, optimizacija proizvodnje. Usmerjena lokalizacija, topnost, stabilnost, renaturacija.
12. Izražanje v kvasovkah, insektnih in sesalskih celicah.
13. Laboratorijska varnost pri delu z GSO.

Praktični del:

1. Načrtovanje začetnih oligonukleotidov, konstruiranje rekombinantnih molekul (računalniška vaja).
2. Preparativno rezanje plazmidne DNA z restriktazami.
3. Izolacija DNA iz gela in ocena koncentracije.
4. Priprava kompetentnih celic.
5. Ligacija fragmentov DNA in transformacija bakterij.
6. Izolacija plazmidne DNA iz transformant v malem merilu.
7. Restrikcijska analiza rekombinantnih vektorskih molekul.
8. Indukcija izražanja rekombinantne DNA.
9. Analiza topnosti rekombinantnega proteina. Lokalizacija rekombinantnega proteina in test biološke aktivnosti.

Seminar:
Ocena tveganja za delo z gensko spremenjenimi organizmi v zaprtem sistemu.

transcription / translation.
11. Expression in prokaryotes: vectors, fusions, optimization of production. Targeted localization, solubility, stability and renaturation.
12. Expression in yeast, insect and mammalian cells.
13. Laboratory safety issues in work with genetically modified organisms.

Practical course:

1. Primer design and construction of recombinant molecules (computer work).
2. Preparative restriction enzyme cleavage of plasmid DNA.
3. DNA isolation from agarose gels and estimation of DNA concentration.
4. Preparation of competent cells.
5. DNA fragment ligation and bacterial transformation.
6. Small-scale plasmid DNA isolation from transformants.
7. Restriction analysis of recombinant vector molecules.
8. Induction of recombinant DNA expression.
9. Solubility assay, localization screening and biological activity test.

Seminar:
Risk assessment for work with GMOs in a contained system.



**Temeljna literatura in viri / Readings:**

- Twyman & Primrose: Principles of Gene Manipulation and Genomics. Oxford: Blackwell Publishing, 2006.
- Dolinar: Molekulsko kloniranje, Navodila za vaje. Ljubljana: Fakulteta za kemijo in kemijsko tehnologijo UL, 2016. Dostopno na spletu: http://www.fkkt.uni-lj.si/fileadmin/datoteke/1-O_fakulteti/7-Zalo%C5%BEba/Skripta_MK_201617.pdf

**Cilji in kompetence:**

Vsak študent mora biti po opravljenem kolokviju in izpitu sposoben ob ustreznem vodstvu sam izvesti osnovne analize DNA, pripraviti rekombinantno molekulo DNA in razumeti osnovne postopke dela pri pripravi

**Objectives and Competences:**

Under guidance, students will be able to perform basic DNA analyses and construct a recombinant DNA molecule. Students will understand fundamental procedures in recombinant protein preparation in different

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| rekombinantnih proteinov v različnih tipih gostiteljskih organizmov. Poznati bo moral tudi načela varnosti dela z gensko spremenjenimi organizmi. | types of host organisms and will be aware of safe laboratory work with genetically modified organisms. |

| **Predvideni študijski rezultati:** | **Intended Learning Outcomes:** |
|---|---|
| Znanje in razumevanje<br>Znanje: poznavanje encimov, ki jih uporabljamo pri delu z DNA in pri pripravi rekombinantnih proteinov, osnove metod za označevanje in analizo nukleinskih kislin, lastnosti vektorskih molekul in metode vnosa DNA v gostiteljsko celico. Zakonska urejenost dela z GSO.<br><br>Razumevanje: postopek PCR, postopek določanja nukleotidnega zaporedja, postopki priprave DNA-knjižnic, načini pridobivanja rekombinantnih proteinov. | Knowledge and Comprehension<br>Knowledge:<br>Knowing enzymes used for DNA modifications and preparation of recombinant proteins, basic methods for nucleic acids labelling and analysis, properties of vector molecules and methods for incorporation of foreign DNA into host cells. Knowing legal framework for working with GMOs.<br><br>Comprehension:<br>PCR technique, DNA sequencing, preparation of DNA libraries, approaches to recombinant protein preparation. |
| Uporaba<br>Razlikovanje med vektorskimi molekulami, občutek za velikosti molekul DNA (bazni pari, masa) in količine (femtomolarno do mikromolarno območje). Izolacija vektorskih DNA iz celic. Rezanje DNA z restriktazami. Ligacija DNA. Transformiranje bakterijskih celic. Biosinteza rekombinantnih proteinov in analiza njegovih lastnosti. | Application<br>Distinguishing between various types of vector molecules, feeling for sizes of biological macromolecules (base pairs vs. molecular mass) and quantities (femtomolar to micromolar range). Isolation of vector molecules from cells. DNA digestion with restriction enzymes. DNA ligation. Bacterial transformation. Biosynthesis of recombinant proteins and their analysis. |
| Refleksija<br>Povezovanje posameznih metod v celoten eksperiment – primer priprave rekombinantnega proteina. Povezovanje dela z DNA z analizo proteinov. | Analysis<br>Combining separate methods into an experiment – case experiments for preparation of a recombinant protein. Work with DNA continues at the protein level. |
| Prenosljive spretnosti<br>Laboratorijsko delo v skupini s kolegom. Pisanje poročil o laboratorijskem delu. Načela varnosti pri laboratorijskem delu z DNA in genetsko spremenjenimi mikroorganizmi. Način priprave ocene tveganja. | Skill-transference Ability<br>Laboratory work in a group with a colleague student. Writing laboratory work reports. Principles of laboratory safety when working with DNA and GM microorganisms. Writing a risk assessment. |

| **Metode poučevanja in učenja:** | **Learning and Teaching Methods:** |
|---|---|
| Predavanja, laboratorijske vaje, individualno delo pri pripravi seminarjev. Spletna gradiva za določena poglavja. | Lectures, laboratory practical courses, individual work for preparing seminars. Web sources for some topics. |

| Načini ocenjevanja: | Delež (v %) / Weight (in %) | Assessment: |
|---|---|---|
| Pisni izpit, seminarska naloga ter ustni kolokvij z vaj.<br>Opravljene vaje so pogoj za pristop k izpitu. | | Written examination, seminary presentation and oral practicals defence.<br>Access to examination only with completed laboratory practicals. |

**Reference nosilca / Lecturer's references:**

- ŠKRLJ, Nives, ERČULJ, Nina, **DOLINAR, Marko**. A versatile bacterial expression vector based on the synthetic biology plasmid pSB1. Protein expression and purification, ISSN 1046-5928, 2009, vol. 64, no. 2, str. 198-204, doi: 10.1016/j.pep.2008.10.019. [COBISS.SI-ID 30190085]
- VASILJEVA, Olga, **DOLINAR, Marko**, ROZMAN PUNGERČAR, Jerica, TURK, Vito, TURK, Boris. Recombinant human procathepsin S is capable of autocatalytic processing at neutral pH in the presence of glycosaminoglycans. FEBS letters, ISSN 0014-5793. [Print ed.], 2005, vol. 579, str. 1285-1290. [COBISS.SI-ID 18842407]
- PUNGERČIČ, Galina, DOLENC, Iztok, **DOLINAR, Marko**, BEVEC, Tadeja, KOKALJ-JENKO, Saša, KOLARIČ, Saša, TURK, Vito. Individual recombinant thyroglobulin type-1 domains are substrates for lysosomal cysteine proteinases. Biological chemistry, ISSN 1431-6730, 2002, vol. 383, str. 1809-1812. [COBISS.SI-ID 17215527]