

UČNI NAČRT PREDMETA / COURSE SYLLABUS

Predmet:	MOLEKULSKO KLONIRANJE
Course Title:	MOLECULAR CLONING

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

Vrsta predmeta / Course Type:

obvezni / Mandatory

Univerzitetna koda predmeta / University Course Code:

BK131

Predavanja Lectures	Seminar Seminar	Vaje Tutorial	Klinične vaje Work	Druge oblike študija	Samost. delo Individual Work	ECTS
30	5	40 LV	/	/	75	5

Nosilec predmeta / Lecturer:

izr. prof. dr. Marko Dolinar /
Dr. Marko Dolinar, Associate Professor

Jeziki / Languages:

Predavanja / Lectures: slovenski / Slovenian

Vaje / Tutorial: slovenski / Slovenian

Pogoji za vključitev v delo oz. za opravljanje študijskih obveznosti:

Študent oz. kandidat mora imeti predmet opredeljen kot študijsko obveznost.

Prerequisites:

The course has to be assigned to the student.

Vsebina:

1. Raziskovanje DNA in molekularna biotehnologija: zgodovina in prihodnost.
2. Laboratorijski organizmi v DNA-tehnologiji.
3. Encimi pri delu z DNA.
4. Elektroforezne metode.
5. Vektorske molekule. Reporterski geni.
6. Sinteza DNA *in vitro*. Sinteza cDNA. Priprava DNA-knjižnic. Transformiranje celic.
7. PCR in izvedene tehnike.
8. Hibridizacija, sonde, načini označevanja DNA. Presejanje knjižnic.
9. Določanje nukleotidnega zaporedja

Content (Syllabus outline):

1. Exploring DNA and molecular biotechnology – past and future.
2. Laboratory organisms in DNA technology.
3. DNA-modifying enzymes.
4. Electrophoretic methods.
5. Vectors and reporters.
6. DNA synthesis *in vitro*. cDNA synthesis. Preparation of DNA libraries. Cell transformation.
7. PCR and deduced techniques.
8. Hybridization, probes and approaches for DNA labelling. Screening of DNA libraries.
9. DNA sequencing.

DNA.

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. Restriksijska 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.

10. Expression systems. In vitro 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: Molekularno 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

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

razumeti osnovne postopke dela pri pripravi rekombinantnih proteinov v različnih tipih gostiteljskih organizmov. Poznati bo moral tudi načela varnosti dela z gensko spremenjenimi organizmi.

recombinant protein preparation in different types of host organisms and will be aware of safe laboratory work with genetically modified organisms.

Predvideni študijski rezultati:

Znanje in razumevanje

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.

Razumevanje: postopek PCR, postopek določanja nukleotidnega zaporedja, postopki priprave DNA-knjižnic, načini pridobivanja rekombinantnih proteinov.

Uporaba

Razlikovanje med vektorskimi molekulami, občutek za velikosti molekul DNA (bazni pari, masa) in količine (femtolarno do mikromolarno območje). Izolacija vektorskih DNA iz celic. Rezanje DNA z restriktazami. Ligacija DNA. Transformiranje bakterijskih celic. Biosinteza rekombinantnih proteinov in analiza njegovih lastnosti.

Refleksija

Povezovanje posameznih metod v celoten eksperiment – primer priprave rekombinantnega proteina. Povezovanje dela z DNA z analizo proteinov.

Prenosljive spretnosti

Laboratorijsko delo v skupini s kolegom. Pisanje poročil o laboratorijskem delu. Načela varnosti pri laboratorijskem delu z DNA in gensko spremenjenimi mikroorganizmi. Način priprave ocene tveganja.

Intended Learning Outcomes:

Knowledge and Comprehension

Knowledge:

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.

Comprehension:

PCR technique, DNA sequencing, preparation of DNA libraries, approaches to recombinant protein preparation.

Application

Distinguishing between various types of vector molecules, feeling for sizes of biological macromolecules (base pairs vs. molecular mass) and quantities (femtolar 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.

Analysis

Combining separate methods into an experiment – case experiments for preparation of a recombinant protein. Work with DNA continues at the protein level.

Skill-transference Ability

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:

Predavanja, laboratorijske vaje, individualno delo pri pripravi seminarjev. Spletna gradiva za določena poglavja.

Learning and Teaching Methods:

Lectures, laboratory practical courses, individual work for preparing seminars. Web sources for some topics.

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Delež (v %) /

Načini ocenjevanja:

Weight (in %)

Assessment:

<p>Pisni izpit, seminarska naloga ter ustni kolokvij z vaj. Opravljene vaje so pogoj za pristop k izpitu.</p>		<p>Written examination, seminary presentation and oral practicals defence. Access to examination only with completed laboratory practicals.</p>
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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]