BIOTEHNOLOGIJA – INDIVIDUALNO RAZISKOVALNI PREDMETI

UČNI NAČRT PREDMETA/COURSE SYLLABUS

Predmet:	Imunološki poskusi in tehnike
Course title:	Immunological experiments and techniques

Študijski programi in stopnja	Študijska smer	Letnik	Semestri
Bioznanosti, tretja stopnja, doktorski	biotehnologija		Celoletni

Univerzitetna koda predmeta/University course code: 3787

Predavanja	Seminar	Vaje	Klinične vaje	Druge oblike študija	Samostojno delo	ECTS
	0	25	0	0	100	5

Nosilec predmeta/Lecturer:	Mojca Narat

Izvajalci predavanj:	Mojca Narat
Izvajalci seminarjev:	
Izvajalci vaj:	Mojca Narat
Izvajalci kliničnih vaj:	
Izvajalci drugih oblik:	
Izvajalci praktičnega usposabljanja:	

Vrsta predmeta/Course type:	individualno raziskovalni /individual research course

Jeziki/Languages:	Predavanja/Lectures:	Angleščina, Slovenščina
	Vaje/Tutorial:	Angleščina, Slovenščina

Pogoji za vključitev v delo oz. za opravljanje študijskih obveznosti:	Prerequisites:
splošni pogoji za vpis na doktorski študij	General prerequisites for enrolment into doctoral studies

Vsebina:	Content (Syllabus outline):
Načrtovanje imunoloških poskusov. Pregled imunoloških metod in tehnik ter izbor tistih, s katerimi se bo kandidat srečeval pri izvedbi doktorskega dela. Pri izvedbi laboratorijskega dela kandidat lahko uporabi svoj material in izvede del doktorske naloge. Možnosti:Pridobivanje poliklonalnih-monoklonalnih-rekombinantnih protiteles. Fagna knjižnica. Konjugiranje protiteles in uporaba za detekcijo/izolacijo/aplikacijo. Izolacija in detekcija antigenov: DIBA, Westrn-blott, ELISA,	Planning of immunological experiments. Overview of immunological methods and techniques and the selection of those that candidate encountered in the implementation of the doctoral dissertation. The candidate can use its own material. Options: Production of monoclonal-polyclonal-recombinant antibodies. Phage display. Conjugation of antibodies and their use for detection / isolation / application. Isolation and detection of antigens: DIBA, Westrn-blott, ELISA, immunoprecipitation. Monitoring of

imunoprecipitacija. Spremljanje imunskega odziva na nivoju molekul (protiteles, citokinov) in izražanja genov. Kompleksni poskusi z uporabo cDNA mikromrež in fenotipskih mikromrež. Celični modeli za proučevanje učinkov antigenov.	immune response at the level of molecules (antibodies, cytokines) and gene expression. Complex experiments using cDNA microarray and phenotypic microarrays. Cell models to study the effects of antigens.

Temeljna literatura in viri/Readings:

Tekoča znanstvena periodika Scientific papers

Cilji in kompetence:	**Objectives and competences:**
Na konkretnem primeru si kandidat sestavi protokol za eksperiment in pridobi kompetence načrtovanja, izvedbe, vrednotenja in prikazovanja rezultatov.	Candidate will design the protocol for experiment and acquire skills of planning, implementation, evaluation and presentation of results.

Predvideni študijski rezultati:	**Intended learning outcomes:**
Znanje in razumevanje: Razumevanje kompleksnosti imunoloških študij. Sposobnost načrtovanja imunoloških poskusov. Konkretna izkušnja dela v imunološkem laboratoriju. Rezultat, ki je del doktorske naloge.	Knowledge and understanding: Understanding the complexity of immunological studies. Ability to design immunological experiments. Practical experience of work in the immunological laboratory. The result, which is part of the doctoral thesis.

Metode poučevanja in učenja:	**Learning and teaching methods:**
Praktično delo v laboratoriju, vodeno s strani mentorice (nosilka predmeta) in konzultacije.	Practical work in the laboratory, supervised by the lecturer and consultations.

Načini ocenjevanja:	**Delež/Weight**	**Assessment:**
Predstavitev rezultatov –preverjanje razumevanja	100,00 %	Presentation of results- verification of understanding

Reference nosilca/Lecturer's references:

1. COLJA VENTURINI, Anja, BRESJANAC, Mara, VRANAC, Tanja, KOREN, Simon, NARAT, Mojca, POPOVIĆ, Mara, ČURIN-ŠERBEC, Vladka. Anti-idiotypic antibodies : a new approach in prion research. *BMC immunology*, ISSN 1471-2172, 2009, vol. 10, article 16, on line.http://www.biomedcentral.com/1471-2172/10/16, doi: 10.1186/1471-2172-10-16. [COBISS.SI-ID 2440072], [JCR, SNIP, WoS do 27. 1. 2014: št. citatov (TC): 6, čistih citatov (CI): 3, normirano št. čistih citatov (NC): 1, Scopus do 14. 1. 2014: št. citatov (TC): 6, čistih citatov (CI): 3, normirano št. čistih citatov (NC): 1]

1. OTA, Katja, LEONARDI, Adrijana, MIKELJ, Miha, SKOČAJ, Matej, WOHLSCHLAGER, Therese, KÜNZLER, Markus, AEBI, Markus, NARAT, Mojca, KRIŽAJ, Igor, ANDERLUH, Gregor, SEPČIĆ, Kristina, MAČEK, Peter. Membrane cholesterol and sphingomyelin, and ostreolysin A are obligatory for pore-formation by a MACPF/CDC-like pore-forming protein, pleurotolysin B. *Biochimie*, ISSN 0300-9084, 2013, vol. 95, iss. 10, str. 1855-1864, doi: 10.1016/j.biochi.2013.06.012. [COBISS.SI-ID 26868007], [JCR, SNIP, WoS do 29. 1. 2014: št. citatov (TC): 1, čistih citatov (CI): 0, normirano št. čistih citatov (NC): 0, Scopus do 8. 1. 2014: št. citatov (TC): 1, čistih citatov (CI): 1, normirano št. čistih citatov (NC): 0]

1. KASTELIC, Saša, BERČIČ, Rebeka Lucijana, CIZELJ, Ivanka, BENČINA, Mateja, MAKRAI, Laszlo, ZORMAN-ROJS, Olga, NARAT, Mojca, BISGAARD, Magne, CHRISTENSEN, Henrik, BENČINA, Dušan. Ornithobacterium rhinotracheale has neuraminidase activity causing desialylation of chicken and turkey serum and tracheal mucus glycoproteins. *Veterinary Microbiology*, ISSN 0378-1135. [Print ed.], 2013,

vol. 162, issues 2-4, str. 707-712, doi: 10.1016/j.vetmic.2012.09.018. [COBISS.SI-ID 3150984], [JCR, SNIP, WoS do 14. 3. 2013: št. citatov (TC): 0, čistih citatov (CI): 0, normirano št. čistih citatov (NC): 0, Scopus do 28. 1. 2013: št. citatov (TC): 0, čistih citatov (CI): 0, normirano št. čistih citatov (NC): 0]

1. CIRKVENČIČ, Nina, NARAT, Mojca, DOVČ, Peter, BENČINA, Dušan. Distribution of chicken cathepsins B and L, cystatin and ovalbumin in extra-embryonic fluids during embryogenesis. *British Poultry Science*, ISSN 0007-1668, 2012, vol. 53, no. 5, str. 623-630, doi:10.1080/00071668.2012.729131. [COBISS.SI-ID 3132296], [JCR, SNIP, WoS do 15. 3. 2013: št. citatov (TC): 0, čistih citatov (CI): 0, normirano št. čistih citatov (NC): 0, Scopus do 15. 1. 2013: št. citatov (TC): 0, čistih citatov (CI): 0, normirano št. čistih citatov (NC): 0]

1. BERČIČ, Rebeka Lucijana, CIZELJ, Ivanka, BENČINA, Mateja, NARAT, Mojca, BRADBURY, Janet M., DOVČ, Peter, BENČINA, Dušan. Demonstration of neuraminidase activity in Mycoplasma neurolyticum and of neuraminidase proteins in three canine Mycoplasma species. *Veterinary Microbiology*, ISSN 0378-1135. [Print ed.], 2012, vol. 155, no. 2/4, str. 425-429. http://pdn.sciencedirect.com/science?_ob=MiamiImageURL&_cid=271229&_user=4776866&_pii=S037811351100472X&_check=y&_origin=browse&_zone=rslt_list_item&_coverDate=2012- 03-23&wchp=dGLzVlk-zSkWb&md5=1bfd4b5e968171ab003546dea1739912/1-s2.0-S037811351100472X-main.pdf, doi: 10.1016/j.vetmic.2011.08.026. [COBISS.SI-ID 2958984], [JCR, SNIP, WoS do 11. 4. 2013: št. citatov (TC): 1, čistih citatov (CI): 0, normirano št. čistih citatov (NC): 0, Scopusdo 6. 2. 2013: št. citatov (TC): 1, čistih citatov (CI): 0, normirano št. čistih citatov (NC): 0]
2. TURKOVÁ, Kristýna, MAVRIČ, Anja, NARAT, Mojca, RITTICH, Bohuslav, ŠPANOVÁ, Alena, ROGELJ, Irena, BOGOVIČ MATIJAŠIĆ, Bojana. Evaluation of Lactobacillus strains for selected probiotic properties. *Folia microbiologica*, ISSN 0015-5632. [Print ed.], 2013, vol. 58, issue 4, str. 261-267, doi: 10.1007/s12223-012-0208-4. [COBISS.SI-ID 3147400]

UČNI NAČRT PREDMETA/COURSE SYLLABUS

Predmet:	Preučevanje bioloških procesov na ravni genoma, transkriptoma in proteoma
Course title:	Global analysis of genome, transcriptome and proteome

Študijski programi in stopnja	Študijska smer	Letnik	Semestri
Bioznanosti, tretja stopnja, doktorski	biotehnologija		Celoletni

Univerzitetna koda predmeta/University course code: 3794

Predavanja	Seminar	Vaje	Klinične vaje	Druge oblike študija	Samostojno delo	ECTS
	7	18	0	0	100	5

Nosilec predmeta/Lecturer: Polona Jamnik

Izvajalci predavanj:	
Izvajalci seminarjev:	Jernej Jakše, Polona Jamnik, Nataša Štajner
Izvajalci vaj:	
Izvajalci kliničnih vaj:	
Izvajalci drugih oblik:	
Izvajalci praktičnega usposabljanja:	

Vrsta predmeta/Course type: individualno raziskovalni /individual research course

Jeziki/Languages:		
	Predavanja/Lectures:	Angleščina, Slovenščina
	Vaje/Tutorial:	Angleščina, Slovenščina

Pogoji za vključitev v delo oz. za opravljanje študijskih obveznosti:	**Prerequisites:**
Splošni pogoji za vpis na doktorski študij.	General conditions for enrollment in doctoral studies.

Vsebina:	**Content (Syllabus outline):**
Izziv današnjega raziskovalnega dela predstavlja povezovanje znanj in eksperimentalnih podatkov posameznih raziskovalnih področij (npr. genomika, transkriptomika, proteomika) in generacija vedno večjih setov podatkov. S tem individualnim raziskovalnim predmetom želimo podati študentu preko laboratorijskih primerov vpogled v konkretne raziskovalne primere in primere obdelav rezultatov s področij genomike, transkriptomike in proteomike: **1) Kvantificiranje DNA/RNA** Kvantificiranje DNA/RNA je obsežno področje, ki se široko uporablja v biotehnoloških raziskavah. Za	The challenge of current research work presents the integration of knowledge and experimental data sets from different research fields (e.g. genomics, transcriptomics, proteomics) and generation of vast data sets. In the frame of this individual research subject we would like to introduce student using real laboratory experiments or data sets with designated research subjects and examples of data analysis from fields of genomics, transcriptomics and proteomics: **1) DNA/RNA quantification**

določevanje tarčnega produkta se tako uporabljajo nekatere tradicionalne metode, kot npr. spektrofotometrija ali PicoGreen dsDNA kvantificiranje, ki niso vrstno specifične, medtem ko je zelo natančno določanje tarčnih genov / DNA / RNA omogočeno predvsem z metodami, ki temeljijo na tehnologiji verižne reakcije. Uporabljajo se v širokem obsegu za DNA kvantificiranje saj amplifikacija tarčne sekvence omogoča visoko občutljivost detekcije. Pri kvantitativnih PCR (QPCR) tehnikah je količina tarčnega gena povezana z intenziteto fluorescence reporterskih molekul. Signal fluorescence na osnovi katerega želimo izračunati začetno količino tarčnega gena lahko merimo na koncu reakcije (endpoint QPCR) ali pa med samim potekom reakcije (real-time QPCR). Novejša tehnologija imenovana digitalni PCR (dPCR) pa je verzija klasičnega PCR-ja, ki se lahko direktno uporablja za kvantificiranje in pomnoževanje nukleinskih kislin. Največja razlika med njima je v tem, da je pri dPCR vzorec razdeljen na veliko število manjših delov v katerih potekajo posamezne reakcije.

V sklopu tega predmeta se bodo študentje seznanili predvsem z uporabo in aplikacijami tehnologije PCR v realnem času, ki je zaenkrat najboj široko uporabna.

2) Obdelava genomskih in transkriptomskih NGS podatkov – uporabna bioinformatika

V zadnjih nekaj letih so postopki naslednjih generacij določevanja nukleotidnih zaporedij (NGS) povsem spremenili področje genomike in transkriptomike. V tem sklopu predmeta se bodo študenti seznanili z naslednjimi aktivnostmi:

a) Hiter vpogled s trenutnimi NGS tehnologijami, ki so aktualne

b) NCBI-jev arhiv »Sequence Read Archive«, čemu je namenjen, prenos surovih podatkov sekvenciranja različnih platform, seznanitev s formati teh podatkov, pretvorba podatkov s pomočjo programskega paketa »SRA Toolkit«

c) Analiza kvalitete NGS podatkov (QC analysis) in interpretacija analize

d) Čiščenje surovih NGS podatkov

e) Osnovni formati NGS podatkov, seznanitev z njimi, njihova obdelava (FASTQ, SAM, BAM, GFF, VCF, BED)

f) *De-novo* zlaganje in rekonstrukcija zaporedij na osnovi mapiranja

g) Vizualizacija NGS podatkov.

3) Preučevanje proteoma

Različni omski pristopi omogočajo preučevanje bioloških procesov na molekularni ravni. Med njimi ima proteomika pomembno prednost, kajti preučuje proteine, ki so nosilci funkcij vsake žive celice. Z uporabo različnih proteomskih orodij lahko pridobimo informacijo o izražanju proteinov,

There are a number of methods available to quantify DNA. The traditional method of DNA quantitation involves measuring the absorbance of the sample on a spectrophotometer. Another method involves the use of a fluorescent dye, is PicoGreen dsDNA quantitation method. But all these techniques are not species-specific, while very precise determination of target genes / DNA / RNA was enabled by methods based on the polymerase chain reaction technology. The latest development in DNA quantitation is based on the technique of real time PCR. Several different approaches of real time quantitation of DNA are based on the principle of fluorescent dye binding double-stranded DNA as it accumulates during the PCR process. As the technique is based on the polymerase chain reaction, DNA quantitation can be undertaken by targeting any specific region of template DNA. Another, improved technology called digital PCR (dPCR), is a refinement of conventional PCR methods that can be used to directly quantify and clonally amplify nucleic acids. dPCR carries out a single reaction within a sample, however the sample is separated into a large number of partitions and the reaction is carried out in each partition individually.

Within this course-set the students will gain the knowledge about most widely applicable real-time PCR methods and techniques.

2) Analysis of genomics and transcriptomics NGS data set – applied bioinformatics

The data sets generated by next generation sequencing methodologies (NGS) revolutionized the fields of genomics and proteomics in the last few years. The students will be familiarized by the next topics:

a) Quick overview of relevant NGS technologies

b) Sequence Read Archive maintained by NCBI, data acquisition produced by different NGS platforms and data formatting using SRA Toolkit

c) QC analysis of raw NGS data

d) NGS data trimming

e) Basic NGS formats, properties, their use (FASTQ, SAM, BAM, GFF, VCF, BED)

f) Read mapping approach of sequence reconstruction and *de-novo* assembly

g) NGS data visualization.

3) Study of proteome

Different omics approaches enable investigation of biological processes at the molecular level. Among them proteomics has an important advantage, it investigates proteins that carry out functions of every living cell. By using different proteomic tools information about protein expression, post-translational modifications and protein interactions can be obtained. In the context of this part students will gain knowledge in the following topics:

njihovih posttranslacijskih modifikacijah in proteinskih interakcijah. Študenti se bodo v tem sklopu praktično seznanili z naslednjimi aktivnostmi: - Priprava biološkega materiala – vzorčenje in priprava vzorca za proteomsko analizo - Analiza proteoma z 2-D elektroforezo - Obdelava 2-D slik gelov z računalniškim programom - Vrednotenje rezultatov identifikacije proteinov, pridobljenih z masno spektroskopijo.	- Biological material preparation – sampling and sample preparation for proteome analysis - Proteome analysis by 2-D electrophoresis - Analysis of 2-D gel images by using specific computer software - Evaluation of protein identification results obtained by mass spectroscopy.

Temeljna literatura in viri/Readings:

1) Real-time PCR handbook. 2012, Real-Time PCR 2nd Edition, Life Technologies Corporation (6 Chapters and 66 pages)
2) Bioinformatics for High Throughput Sequencing. Rodríguez-Ezpeleta, Naiara, Hackenberg, Michael, Aransay, Ana M. (Eds.) 2012, XI, 255p. 29 illus., 25 illus. in color.

3) Revialni in originalni znanstveni članki s področja/Review and original scientific articles from the field.

Cilji in kompetence:	**Objectives and competences:**
Namen predmeta je: 1) predstaviti tehnike kvantificiranja DNA/RNA ter jih podpreti s konkretnimi laboratorijskimi poskusi in izračuni ter vrednotenji dobljenih rezultatov, 2) seznaniti študente z osnovnimi karakteristikami podatkov NGS, njihovimi oblikami, podatkovnimi bazami za shranjevanje, ter s potekom analize. 3) predstaviti analizo proteoma od priprave vzorca, separacije proteinov do vrednotenja proteomskih podatkov Študenti bodo preko praktičnih primerov spoznali, kako razumeti biološke procese na ravni genoma, transkriptoma in proteoma in znali pravilno načrtovati eksperiment.	The objective of this course is to: 1) introduce the DNA/RNA quantification methods and to perform some practical laboratory experiments and calculations on the basis of the obtained results, 2) acquaint students with basics characteristics of NGS data, their databases for storing and with recommended flow of the analysis 3) introduce proteome analysis from sample preparation , protein separation to proteomic data evaluation Based on the practical examples and real data sets students will learn how to understand biological processes on the level of genome, transcriptome and proteome. They will also be able to properly design such experiments.

Predvideni študijski rezultati:	**Intended learning outcomes:**
Znanje in razumevanje: • Pridobitev znanja s področja postavitve poskusa za PCR v realnem času • Razumevanje in poznavanje metod in tehnik za določanje količine tarčnega gena v vzorcu oz. izražanje posameznih tarč z metodo PCR v realnem času. • Analiza podatkov in vrednotenje rezultatov z različnimi metodološkimi pristopi. • Statistična analiza rezultatov in grafična predstavitev • Poznavanje osnov uporabne bioinformatike na primerih NGS podatkov	Knowledge and understanding: • To gain knowledge on basic principles of Real-Time PCR assay design • To understand methods and techniques for determining the amount of target gene in the sample or analyzing the expression of individual targets by the method of real-time PCR. • Data analysis and evaluation of the results of different methodological approaches. • Statistical analysis of results and graphical presentation • Understanding of the basis of applied bioinformatics related to NGS data

• Poznavanje pomena proteomike za razumevanje bioloških procesov in sposobnost načrtovanja proteomskega eksperimenta od priprave biološkega materiala do analize proteoma z 2-D elektroforezo in obdelavo podatkov.	• Knowledge of importance of proteomics for understanding biological processes and ability to design proteomic experiment from biological material preparation to proteome analysis by 2-D electrophoresis and data evaluation.

Metode poučevanja in učenja:	**Learning and teaching methods:**
• Teoretične osnove • Praktično laboratorijsko delo oz. delo z računalnikom • Analiza rezultatov s pomočjo programske opreme in različnih računalniških aplikacij	• Theoretical basic • Practical lab work or computer work • Analysis of the results using specialized computer software

Načini ocenjevanja:	**Delež/Weight**	**Assessment:**
Izdelava in predstavitev projektne naloge	100,00 %	Research and presentation of project work

Reference nosilca/Lecturer's references:

Nataša Štajner

1. ŠTAJNER, Nataša, JAVORNIK CREGEEN, Sara Joan, JAVORNIK, Branka. Evaluation of reference genes for RT-qPCR expression studies in hop (Humulus lupulus L.) during infection with vascular pathogen Verticillium albo-atrum. PloS one, ISSN 1932-6203, 2013, vol. 8, issue 7, str. 1-13 (e68228), ilustr.http://dx.doi.org/10.1371/journal.pone.0068228, doi: 10.1371/journal.pone.0068228. [COBISS.SI-ID 7653753]
2. REŠETIČ, Tjaša, ŠTAJNER, Nataša, BANDELJ MAVSAR, Dunja, JAVORNIK, Branka, JAKŠE, Jernej. 2. Validation of candidate reference genes in RT-qPCR studies of developing olive fruit and expression analysis of four genes involved in fatty acids metabolism. Molecular breeding, ISSN 1380-3743. [Tiskana izd.], 2013, vol. 32, issue 1, str. 211-222.http://dx.doi.org/10.1007/s11032-013-9863-7, doi: 10.1007/s11032-013-9863-7. [COBISS.SI-ID 7527801]
3. ORAŽEM, Petra, ŠTAJNER, Nataša, BOHANEC, Borut. Effect of X-ray irradiation on olive shoot culture evaluated by morphological measurements, nuclear DNA content and SSR and AFLP markers. Trees, ISSN 0931-1890, 2013, vol. 27, issue 6, str. 1587-1595, ilustr. http://link.springer.com/content/pdf/10.1007%2Fs00468-013-0906-9.pdf, doi:10.1007/s00468-013-0906-9. [COBISS.SI-ID 7652217]
4. JAKŠE, Jernej, ŠTAJNER, Nataša, LUTHAR, Zlata, JELTSCH, Jean-Marc, JAVORNIK, Branka. Development of transcript-associated microsatellite markers for diversity and linkage mapping studies in hop (Humulus lupulus L.). Molecular breeding, ISSN 1380-3743. [Tiskana izd.], 2011, vol. 28, no. 2, str. 227-239. http://dx.doi.org/10.1007/s11032-010-9476-3, doi: 10.1007/s11032-010-9476-3. [COBISS.SI-ID 6353273]
5. RUSJAN, Denis, JUG, Tjaša, ŠTAJNER, Nataša. Evaluation of genetic diversity: which of the varieties can be named 'Rebula' (Vitis vinifera L.)?. Vitis, ISSN 0042-7500, 2010, vol. 49, no. 4, str. 189-192. [COBISS.SI-ID 6464889]
6. ŠTAJNER, Nataša, ŠATOVIĆ, Zlatko, ČERENAK, Andreja, JAVORNIK, Branka. Genetic structure and differentiation in hop (Humulus lupulus L.) as inferred from microsatellites. Euphytica, ISSN 0014-2336. [Print ed.], 2008, vol. 161, no. 1-2, str. 301-311. http://dx.doi.org/10.1007/s10681-007-9429-z, doi: 10.1007/s10681-007-9429-z. [COBISS.SI-ID 5469817]
7. JAKŠE, Jernej, ŠTAJNER, Nataša, KOZJAK, Petra, ČERENAK, Andreja, JAVORNIK, Branka. Trinucleotide microsatellite repeat is tightly linked to male sex in hop (Humulus lupulus L.). Molecular breeding, ISSN 1380-3743. [Tiskana izd.], 2008, vol. 21, no. 2, str. 139-148. [COBISS.SI-ID 5111417]
8. ŠTAJNER, Nataša, KOROŠEC-KORUZA, Zora, RUSJAN, Denis, JAVORNIK, Branka. Microsatellite genotyping of old Slovenian grapevine varieties (Vitis viniferaL.) of the Primorje (coastal) winegrowing region. Vitis, ISSN 0042-7500, 2008, vol. 47, no. 4, str. 201-204. [COBISS.SI-ID 5704825]

Polona Jamnik

1. PETELINC, Tanja, POLAK, Tomaž, **JAMNIK, Polona**. Insight into the molecular mechanisms of propolis activity using a subcellular proteomic approach. *Journal of agricultural and food chemistry*, ISSN 0021-8561, 2013, vol. 61, str. 11502-11510, doi: 10.1021/jf4042003. [COBISS.SI-ID 4320376]
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