<table>
<tr><td colspan="2">NAME OF THE COURSE</td><td colspan="5">Epigenetics</td></tr>
<tr><td>Code</td><td>PMB710</td><td>Year of study</td><td colspan="4">1.</td></tr>
<tr><td>Course teacher</td><td>Željana Fredotović, PhD, Assistant Professor</td><td>Credits (ECTS)</td><td colspan="4">3,0</td></tr>
<tr><td rowspan="2">Associate teachers</td><td rowspan="2"></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>15</td><td></td><td>30</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">30%</td></tr>
<tr><td colspan="6">COURSE DESCRIPTION</td></tr>
<tr><td>Course objectives</td><td colspan="5">To provide students with fundamental theoretical understanding of basic and complex epigenetic phenomena. Explore the molecular mechanisms underlaying epigenetic phenomena. Introduce students with the importance of epigenetics and its impact on human and animal development, evolution and human disease.</td></tr>
<tr><td>Course enrolment requirements and entry competences required for the course</td><td colspan="5">Cell biology, Genetics, Molecular biology</td></tr>
<tr><td>Learning outcomes expected at the level of the course (4 to 10 learning outcomes)</td><td colspan="5">Students will:
• Know the basic mechanisms included in epigenetic changes
• Know how the environment causes epigenetic changes
• Understand the role of epigenetics in gene regulation and disease
• Gain practical skills in performing epigenetic experiments on gene regulation</td></tr>
<tr><td>Course content broken down in detail by weekly class schedule (syllabus)</td><td colspan="5">Lectures:
• Introduction to epigenetics, basic concepts and brief history. Chromatin, histone code, epigenetic modifications and gene expression: DNA methylation, acetylation. Linking epigenetic modifications to chromatin remodelling and transcription. Long non-coding RNAs, microRNA, piRNAs in epigenetics (3 hours)
• Dosage compensation: X chromosome inactivation in mammals, history and background on X-inactivation, random inactivation of X chromosome, X-inactivation by genomic imprinting, regulation of X-inactivation, epigenetic mechanisms of X chromosome inactivation, maintains of silencing. Dosage compensation in flies and worms, compared with mammals (3 hours)
• Genomic imprinting and epigenetic reprogramming: introduction to epigenetic reprogramming of the maternal and paternal genome. Evolution of imprinted genes. Identification of imprinted genes and their location on chromosome. The role of DMR, methylation, ncRNA in genomic imprinting (3 hours)
• Epigenetics and the environment: Nature vs. Nurture. Examples of the influence of the environment on epigenome. Nutrition and epigenome. Behaviour and epigenome. Toxins and epigenome. Mechanisms of environmental influence on epigenetic control and transgenerational epigenetic inheritance through the gametes (3 hours)</td></tr>
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- Epigenetics in cancer and disease (3 hours)

Laboratory exercises (30 hours):
Mini project no.1

- Students will be given sequence-identical lambda DNA samples, one of which contains methylations, while other does not. These two lambda samples should present different restriction patterns after digestion with methylation-sensitive restriction enzyme such as *Dpn*I.
- The cleavage of lambda DNA with restriction enzyme *Hind*III or *Dpn*I was carried out as described in the instruction manual for the restriction enzyme (positive controls). Negative controls consisted of methylated and non-methylated lambda DNA, treated as described in manual, but without addition of the restriction enzyme.
- Preparation if agarose gel and pipeting each restriction sample into gel slots. Running of the gel at 100 V.
- Visualisation of DNA fragments and analysis.

Mini project no.2

- Students design two sets of primers and performing a bisulfide conversion. It is relatively easy to detect the methylation level of a specific region of a gene (the promoter is usually a good place to start). The bisulfide conversion reaction converts all unmethylated cytosines into uracil nucleotides. One set of primers matches the DNA, and the other set of primers matches the DNA after all the methylated nucleotides are converted using the bisulfide conversion method. As the conversion reaction changes the annealing temperature of the template (cytosine supports three hydrogen bonds, but uracil supports only two hydrogen bonds), only one of these sets of primers should bind effectively. The primer set that amplifies the target sequence faster indicates if the cytosines in the target gene are methylated. The target gene for this analysis is *Per1* gene, one of the many that control circadian rhythms, that demonstrate the effects of methylation. Genes in this family encode components of the circadian rhythms of locomotor activity, metabolism, and behaviour.
    - Conversion of methylated nucleotides was carried out using the EZ DNA Methylation-Direct Kit from Zymo Research Corp.
- RNA isolation from the cheek cells
- cDNA synthesis
- qPCR analysis
- Analysis of gene expression level may be carried out through gel electrophoresis or through qPCR.
- For each sample, two QPCRs were run—one with a set of primers that will anneal to DNA that has not undergone bisulfide conversion and one with a set of primers designed to anneal to DNA that has done so. Analysis of the gene's methylation levels may be carried out by agarose gel electrophoresis (see relevant section later) or with a comparison of the samples' Cts (The sample tested with primers that were designed to anneal to DNA that had not undergone bisulfide conversion had a lower Ct, 24.44, than the converted sample Ct of 33.61. This indicates that less amplification of the

	converted sample (where methylated cytosines are converted to uridines) was achieved because the primers could not anneal precisely to the template DNA, and therefore, there was relatively little methylation.					
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 presence in the amount of at least 70% of scheduled lectures. Performed all laboratory exercises.					
Screening student work *(name the proportion of ECTS credits for each activity so that the total number of ECTS credits is equal to the ECTS value of the course)*	Class attendance	0,5	Research		Practical training	
	Experimental work	0,5	Report		(Other)	
	Essay		Seminar essay		(Other)	
	Tests		Oral exam		(Other)	
	Written exam	2	Project		(Other)	
Grading and evaluating student work in class and at the final exam	Grading will be conducted based on activities in class, practical exercises in the laboratory, and the final written exam.					

	Title	**Number of copies in the library**	**Availability via other media**
Required literature (available in the library and via other media)	Allis C.D., Jenuwein T., Reinberg D., Caparros M-L. Epigenetics. 2007. Cold Spring Harbor Laboratory Press. Cold Spring Harbor, NY.		
	Trygve O. Tollefsbol (auth.), Trygve O. Tollefsbol (eds.) 2011. Epigenetics protocols. Human Press.		
	Detailed handouts provided by the instructor		

Optional literature (at the time of submission of study programme proposal)	Original and review scientific articles.
Quality assurance methods that ensure the acquisition of exit competences	Students questionnaire.
Other (as the proposer wishes to add)	