GDAŃSK UNIVERSITY OF TECHNOLOGY

Subject card

Subject name and code	Techniques of Amplification of Nucleic Acids, PG_00058269		
Field of study	Biotechnology		
Date of commencement of studies	February 2023	Academic year of realisation of subject	2023/2024
Education level	second-cycle studies	Subject group	Optional subject group Subject group related to scientific research in the field of study
Mode of study	Full-time studies	Mode of delivery	at the university
Year of study	1	Language of instruction	Polish
Semester of study	2	ECTS credits	5.0
Learning profile	general academic profile	Assessment form	exam
Conducting unit	Department of Microbiology -> Faculty of Chemistry		
Name and surname of lecturer (lecturers)	Subject supervisor	dr hab. Beata Krawczyk	
	Teachers	dr hab. Beata Krawczyk	

Lesson types and methods of instruction

Lesson type	Lecture	Tutorial	Laboratory	Project	Seminar	SUM
Number of study hours	30.0	0.0	30.0	0.0	0.0	60

E-learning hours included: 0.0

Learning activity and number of study hours

Learning activity	Participation in didactic classes included in study plan	Participation in consultation hours	Self-study	SUM
Number of study hours	60	10.0	55.0	125

Subject objectives

The aim of the course is to acquaint the student with the techniques of nucleic acid amplification, which can be used as a basic tool for medical diagnosis, food and biotechnology

Learning outcomes

Course outcome	Subject outcome	Method of verification
[K7_K04] is aware of the need to solve problems and perform tasks, independently formulate questions to solve a given problem or task; is able to plan the execution of a larger task by dividing it into partial tasks and draw up an appropriate schedule	The student understands that nucleic acid amplification techniques require optimisation. The student is able to carry out this process.	[SK5] Assessment of ability to solve problems that arise in practice
[K7_W01] has advanced knowledge of methods of genetic engineering and molecular genetics, functioning of the immune system and mechanisms of immune system response, diagnostic methods, and analytical methods in the area of specialty	Knowledge of different nucleic acid amplification techniques, ability to choose the appropriate one for the intended purpose	[SW3] Assessment of knowledge contained in written work and projects
[K7_U05] is able to apply instrumental methods of quantitative and qualitative analysis and studies on activity of biomolecules, select and apply diagnostic and analytical methods in the field of his/her specialty with particular emphasis on genetic, molecular and microbiological diagnostics and diagnostics based on antigen-antibody reaction	the student is able to select an appropriate methodology and type of polymerase for the planned reaction in order to achieve the desired effect	[SU4] Assessment of ability to use methods and tools

Subject contents	Lectures: Advantages of nucleic acid-based tests (NAT). Target amplification methods. Laboratory organization of nucleic acid amplification. History of PCR. Understanding PCR: how does PCR work, the kinetics of PCR, getting started, post-PCR analysis. Basic PCR reagents and instrumentation: nucleic acid templates, reagents (dNTP, Mg), PCR buffers, nucleotides, oligonucleotide primers, DNA polymerases for PCR (properties). Template DNA preparation and inhibitors of PCR. Optimization of PCR. Troubleshooting, substances affecting PCR: inhibition or enhancement. HOT-start PCR. Touchdown PCR. Advantages and limitation (contamination problems and preventing contamination, control reactions). Improving specificity of PCR. Nested PCR improves PCR sensitivity. Multiplex PCR analysis. Asymetric PCR for generating ssDNA for sequencing. Analysis of gene expression by RT-PCR (reverse transcriptase PCR). Semi-quantitative and quantitative RT-PCR. Competitive PCR. Allele-specific amplification. .Rapid amplification of cDNA ends (RACE). T-RFLP method to study environmental microbes. Real-time PCR basic principles. Genotyping by Random Amplified Polymorphic DNA PCR (RAPD). Ligation mediate PCR Methods (LM PCR). LAMP method. Variable number tandem repeats in identity testing. Application PCR in molecular diagnostics. Alternative methods for target amplification (NASBA, TMA, SDA, MDA,OLA). The use of polymerase Phi29. Signal amplification methods (bDNA branched DNA, hybryd capture assay) and probes amplification methods. Laboratories: Student understands the principles of action PCR, discuss the theory and components of the PCR (explains: a cyclic, expotential, in vitro amplification process, temperature and time profile of thermal cycling, mechanism of action DNA polymerase) and general application of PCR. Student understand problem of contamination and falsely interpreted results. Students can prepare of PCR - adds suitable components. Student is aware of the potential contamination of the sample. Student is able to optimize the PCR reaction. Student understands the problem and can solve problems when there is no PCR product, multiple non-specific products are generated, smeared products are generated, there is faint product band. Student is able to design primers. Student can use the software and databases to design primers. Student can design and optimize Multiplex PCR. Student can be working in micro scale with precision. How Phi29 polymerase works to replicate total DNA
Prerequisites and co-requisites	Passed exams: Microbiology, Molecular biology

Assessment methods and criteria

Subject passing criteria	Passing threshold	Percentage of the final grade
lecture - pass exam	60.0%	50.0%
laboratories - report, test	60.0%	50.0%

Recommended reading

Basic literature	1. Krawczyk B., Kur J. Diagnostyka molekularna w mikrobiologii. Podręcznik, Wyd. Politechnika Gdańska, ISBN 978-83-7348-237-1. 2. Krawczyk B., Kotłowski R., Stojowska K., Szemiako K. Podstawy Techniki PCR - ćwiczenia laboratoryjne.
Supplementary literature	1. PCR: M.McPherson & S.Moller; 2006; 2.PCR: Clinical Diagnostics and Research. A. Rolfs, I. Schuller, U. Finckh, I. Weber-Rolfs; 3.Roche Applied Science; 4. Kalkulacja Tm dla starterów: http://www.roche-applied-science.com/blenchmate; 5. http://www.invitrogen.com; 6. http://blast.ncbi.nlm.nih.gov; 7. http://insilico.ehu.es/ 8. htpp://molbiol-tools.ca/PCR.htm
eResources addresses	Adresy na platformie eNauczanie:

Example issues/ example questions/ tasks being completed	1. What determines the efficiency of the amplification reaction by PCR? 2. Troubleshooting PCR.
Work placement	Not applicable