MINISTRY OF HEALTH OF UKRAINE
ODESA NATIONAL MEDICAL UNIVERSITY

Medical, International Faculty

Department of General and Clinical Epidemiology and Biosafety

with course in Microbiology and Virology

Syllabus of course
**"WHOLE-GENOME SEQUENCING OF BACTERIA.
THEORY AND APPLICATION"**

Volume:	Total number of hours: 90 hours, 3 credits Semester: III – IV 2nd course
Days, Time, Place:	According to the Schedule Department of General and Clinical Epidemiology and Biosafety with course in Microbiology and Virology, academic discipline "Microbiology, Virology and Immunology". Odesa, 1 Knyazivska str., rooms 1-6
Teacher(s)	Hruzesvkyi O.A., MD, Doctor of Science, full professor; Associate professors: Holovatiuk O.L., MD, PhD, Koltsova I.G., MD, PhD, Kurtova M.M., MD, PhD, Shevchuk H.Y., PhD; Assistant professors: Denysko T.V., Dubina A.V, MD, Kahliak M.D., Tabulina A.M., Tarasov Y.V., MD
Contact information	*Phone:* Shevchuk Hanna, Head of studies 093-419-96-77 Dubina Anzhela, responsible for the organizational and educational work of the department 067-428-63-43 Cheban Maya, laboratory assistant 048-753-09-81 E-mail: onmedumicrobio@onmedu.edu.ua; Offline consultations: Thursday – 14.00 - 16.00; Saturday – 9.00 - 13.00; Online consultations: Thursday – 14.00 - 16.00; Saturday – 9.00 - 13.00; The link to the online consultation is provided to each group during the classes separately.

COMMUNICATION

Communication with students will be carried out in the classroom (in person).
During distance learning, communication is carried out through the Microsoft Teams platform, Moodle, as well as through e-mail correspondence, Viber and Telegram messengers (through groups created in Telegram for each group, separately through the group head).

ANNOTATION OF THE COURSE

The subject of study of the discipline is modern molecular genetic research. The course will introduce students to the basic principles of DNA/RNA extraction, as well as classical and quantitative PCR, and will reveal some of the nuances associated with sequencing technologies, post-sequencing data pre-processing procedures, and genome assembly methods (using prokaryotes as an example).

The course also covers the basic concepts and tools of metagenomics, as well as its use in clinical practice.

Prerequisites and post-requisites of the discipline (place of the discipline in the educational program):

Prerequisites: Ukrainian language (for professional purposes), foreign language (for professional purposes), Latin and medical terminology, medical biology, medical and biological physics, biological and bioorganic chemistry, human anatomy, histology, cytology and embryology, physiology, microbiology, virology and immunology, pathological physiology, pathological anatomy, clinical immunology and allergology.

Post-requisites: epidemiology, infectious diseases with pediatric infectious diseases, internal medicine other clinical disciplines.

The purpose is: to master the knowledge and skills in the basic methods of molecular genetic analysis, formation of a general understanding of NGS, principles and stages, use in microbiology and clinical practice.

The tasks of the discipline:
1.   To introduce students to modern sequencing methods.
2.   To introduce students to the main data formats after sequencing and methods of working with them.
3.   To teach to analyze the quality of the obtained sequencing data using cloud algorithms.
4.   To teach to plan and conduct research in the field of bionformatics with NGS data.
5.   To teach algorithms for assembling genomes (for example, prokaryotes).
6.   To introduce the basics of bioinformatic analysis of the assembled genome.
7.   To introduce students to the challenges posed by the need to study complex microbial communities to healthcare professionals.
8.   To introduce students to software products and analytical platforms designed to work with genomic data.

Expected result:
As a result of studying the discipline, the student has to:
Know:
- principles and mechanisms of polymerase chain reaction and its modifications;
- basic principles of various NGS technologies;
- procedure and principles of genome assembly;
- theory and principles of microbial metagenomics.
Be able:
- choose the optimal molecular genetic research methods depending on the task;
- correctly select and use methods for bioinformatic processing of nucleic acid sequencing results.

DESCRIPTION OF THE COURSE

Forms and methods of teaching
The course will be presented in the form of practical lessons (30 hours), organization of independent work of students (60 hours).

Teaching methods: conversation, explanation, discussion, discussion of the acute issues; visual methods: illustration (including multimedia presentations); testing.

The content of the discipline
Theme 1: Basics of molecular biology.
Theme 2. Polymerase chain reaction (PCR).
Theme 3. Real-time PCR and other modifications.
Theme 4. DNA and RNA sequencing. Technologies and platforms for NGS (next generation sequencing).
Theme 5. NGS. Standard applications and methods.

Theme 6. Technologies for pre-processing of next-generation sequencing data.

Theme 7. Principles of DNA sequence assembly.

Theme 8: PATRIC - a relational database and bioinformation resource focused on genomics, designed to help scientists in the study of infectious diseases.

Theme 9: Tools of the Center for Genomic Epidemiology (CGE).

Theme 10. Whole genome sequencing. Serotyping of Salmonella and Escherichia coli strains, as well as the detection of plasmid replicons.

Theme 11: Analysis of 16S rRNA gene sequences for the identification of bacteria in clinical microbiology and infectious diseases.

Theme 12: Metagenomics. Basic approaches.

Theme 13. Resources for analyzing metagenomic data.

Theme 14. Metagenomics in the surveillance of pathogens and antimicrobial resistance.

Theme 15: Achievements and trends in the development of "omics" technologies.

List of recommended literature:
Main:

1. David P. Clark, Nanette J. Pazdernik, Michelle R. McGehee. Molecular Biology, Third Edition. (Elsevier Ltd, 2019), https://doi.org/10.1016/C2015-0-06229-3
2. Hirak Ranjan Dash, Kelly M. Elkins and Noora Rashid Al-Snan, Next Generation Sequencing (NGS) Technology in DNA Analysis, Elsevier Inc. 2023, https://doi.org/10.1016/C2021-0-01718-1

Additional:

3. Zhu H, Zhang H, Xu Y, Laššáková S, Korabečná M, Neužil P. PCR past, present and future. Biotechniques. 2020 Oct;69(4):317-325. doi: 10.2144/btn-2020-0057. Epub 2020 Aug 20. PMID: 32815744; PMCID: PMC7439763.
4. Garibyan L, Avashia N. Polymerase chain reaction. J Invest Dermatol. 2013 Mar;133(3):1-4. doi: 10.1038/jid.2013.1. PMID: 23399825; PMCID: PMC4102308.
5. Quan PL, Sauzade M, Brouzes E. dPCR: A Technology Review. Sensors (Basel). 2018 Apr 20;18(4):1271. doi: 10.3390/s18041271. PMID: 29677144; PMCID: PMC5948698.
6. Valones MA, Guimarães RL, Brandão LA, de Souza PR, de Albuquerque Tavares Carvalho A, Crovela S. Principles and applications of polymerase chain reaction in medical diagnostic fields: a review. Braz J Microbiol. 2009 Jan;40(1):1-11. doi: 10.1590/S1517-83822009000100001. Epub 2009 Mar 1. PMID: 24031310; PMCID: PMC3768498.
7. Teymouri M, Mollazadeh S, Mortazavi H, Naderi Ghale-Noie Z, Keyvani V, Aghababaei F, Hamblin MR, Abbaszadeh-Goudarzi G, Pourghadamyari H, Hashemian SMR, Mirzaei H. Recent advances and challenges of RT-PCR tests for the diagnosis of COVID-19. Pathol Res Pract. 2021 May;221:153443. doi: 10.1016/j.prp.2021.153443. Epub 2021 Apr 14. PMID: 33930607; PMCID: PMC8045416.
8. Goodwin S, McPherson JD, McCombie WR. Coming of age: ten years of next-generation sequencing technologies. Nat Rev Genet. 2016 May 17;17(6):333-51. doi: 10.1038/nrg.2016.49. PMID: 27184599; PMCID: PMC10373632.
9. van Dijk EL, Jaszczyszyn Y, Thermes C. Library preparation methods for next-generation sequencing: tone down the bias. Exp Cell Res. 2014 Mar 10;322(1):12-20. doi: 10.1016/j.yexcr.2014.01.008. Epub 2014 Jan 15. PMID: 24440557.
10. van Dijk EL, Jaszczyszyn Y, Naquin D, Thermes C. The Third Revolution in Sequencing Technology. Trends Genet. 2018 Sep;34(9):666-681. doi: 10.1016/j.tig.2018.05.008. Epub 2018 Jun 22. PMID: 29941292.
11. Sun X, Song L, Yang W, Zhang L, Liu M, Li X, Tian G, Wang W. Nanopore Sequencing and Its Clinical Applications. Methods Mol Biol. 2020;2204:13-32. doi: 10.1007/978-1-0716-0904-0_2. PMID: 32710311.
12. Moran-Gilad J, Yagel Y (ed), Application and integration of omics-powered diagnostics in clinical and public health microbiology. Springer, Cham, Switzerland.

13. Gautam SS, Kc R, Leong KW, Mac Aogáin M, O'Toole RF. A step-by-step beginner's protocol for whole genome sequencing of human bacterial pathogens. J Biol Methods. 2019 Mar 15;6(1):e110. doi: 10.14440/jbm.2019.276. PMID: 31453259; PMCID: PMC6706130.

14. Quainoo S, Coolen JPM, van Hijum SAFT, Huynen MA, Melchers WJG, van Schaik W, Wertheim HFL. Whole-Genome Sequencing of Bacterial Pathogens: the Future of Nosocomial Outbreak Analysis. Clin Microbiol Rev. 2017 Oct;30(4):1015-1063. doi: 10.1128/CMR.00016-17. Erratum in: Clin Microbiol Rev. 2017 Nov 1;31(1): PMID: 28855266; PMCID: PMC5608882.

15. Uelze L, Grützke J, Borowiak M, Hammerl JA, Juraschek K, Deneke C, Tausch SH, Malorny B. Typing methods based on whole genome sequencing data. One Health Outlook. 2020 Feb 18;2:3. doi: 10.1186/s42522-020-0010-1. PMID: 33829127; PMCID: PMC7993478.

16. Fricke, W. Florian & Rasko, David. (2013). Bacterial genome sequencing in the clinic: Bioinformatic challenges and solutions. Nature reviews. Genetics. 15. 10.1038/nrg3624.

17. Bogaerts B, Winand R, Van Braekel J, Hoffman S, Roosens NHC, De Keersmaecker SCJ, Marchal K, Vanneste K. Evaluation of WGS performance for bacterial pathogen characterization with the Illumina technology optimized for time-critical situations. Microb Genom. 2021 Nov;7(11):000699. doi: 10.1099/mgen.0.000699. PMID: 34739368; PMCID: PMC8743554.

18. Ellington MJ, Ekelund O, Aarestrup FM, Canton R, Doumith M, Giske C, Grundman H, Hasman H, Holden MTG, Hopkins KL, Iredell J, Kahlmeter G, Köser CU, MacGowan A, Mevius D, Mulvey M, Naas T, Peto T, Rolain JM, Samuelsen Ø, Woodford N. The role of whole genome sequencing in antimicrobial susceptibility testing of bacteria: report from the EUCAST Subcommittee. Clin Microbiol Infect. 2017 Jan;23(1):2-22. doi: 10.1016/j.cmi.2016.11.012. Epub 2016 Nov 23. PMID: 27890457.

19. Austin-Tse CA, Jobanputra V, Perry DL, Bick D, Taft RJ, Venner E, Gibbs RA, Young T, Barnett S, Belmont JW, Boczek N, Chowdhury S, Ellsworth KA, Guha S, Kulkarni S, Marcou C, Meng L, Murdock DR, Rehman AU, Spiteri E, Thomas-Wilson A, Kearney HM, Rehm HL; Medical Genome Initiative*. Best practices for the interpretation and reporting of clinical whole genome sequencing. NPJ Genom Med. 2022 Apr 8;7(1):27. doi: 10.1038/s41525-022-00295-z. PMID: 35395838; PMCID: PMC8993917.

20. Clarridge JE 3rd. Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. Clin Microbiol Rev. 2004 Oct;17(4):840-62, table of contents. doi: 10.1128/CMR.17.4.840-862.2004. PMID: 15489351; PMCID: PMC523561.

21. Johnson, J.S., Spakowicz, D.J., Hong, BY. et al. Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis. Nat Commun 10, 5029 (2019). https://doi.org/10.1038/s41467-019-13036-1

22. Dai X, Shen L. Advances and Trends in Omics Technology Development. Front Med (Lausanne). 2022 Jul 1;9:911861. doi: 10.3389/fmed.2022.911861. PMID: 35860739; PMCID: PMC9289742.

23. Karczewski KJ, Snyder MP. Integrative omics for health and disease. Nat Rev Genet. 2018 May;19(5):299-310. doi: 10.1038/nrg.2018.4. Epub 2018 Feb 26. PMID: 29479082; PMCID: PMC5990367.

24. Committee on the Review of Omics-Based Tests for Predicting Patient Outcomes in Clinical Trials; Board on Health Care Services; Board on Health Sciences Policy; Institute of Medicine; Micheel CM, Nass SJ, Omenn GS, editors. Evolution of Translational Omics: Lessons Learned and the Path Forward. Washington (DC): National Academies Press (US); 2012 Mar 23. 2, Omics-Based Clinical Discovery: Science, Technology, and Applications. Available from: https://www.ncbi.nlm.nih.gov/books/NBK202165/

CRITERIA EVALUATION

Ongoing control: individual survey on the theme, testing, evaluation of practical skills,

solving situational problems, the ability to analyze and interpret research results and correctly draw reasonable conclusions, evaluation of activity in the classroom.

Criteria of ongoing assessment at the practical class

Score	Assessment criterion
Excellent «5»	The student takes an active part in practical classes, demonstrates deep knowledge, gives complete and detailed answers to questions. Takes an active part in discussing problem situations, demonstrates good skills and abilities in performing practical tasks, correctly evaluates the results. Test tasks are completed in full.
Good «4»	The student participates in practical classes; has a good command of the material. Demonstrates the necessary knowledge, but answers questions with some mistakes; participates in the discussion of problem situations. Test tasks are completed in full, at least 70% of answers to questions are correct.
Satisfactory «3»	The student sometimes participates in practical classes; partially speaks and asks questions; makes mistakes when answering questions; shows passive work in practical classes. Demonstrates skills and abilities in performing practical tasks, but evaluates the results obtained insufficiently fully and accurately. Testing is completed in full, at least 50% of answers are correct, answers to open questions are not logical, with obvious significant errors in definitions.
Unsatisfactory «2»	The student does not participate in the practical lesson, is only an observer; never speaks and does not ask questions, is not interested in learning the material; gives incorrect answers to questions, demonstrates insufficient skills and abilities, cannot cope with practical work and evaluation of the results. Testing is not completed.

Final control: Credit is given to an applicant who has completed all the tasks of the work program of the discipline, actively participated in seminars and has an average current grade of at least 3.0 and has no academic debt.

Possibility and conditions for receiving additional (bonus) points: not provided.

INDEPENDENT WORK OF STUDENTS

Independent work involves preparation for each seminar, independent study of a certain list of topics or topics that require in-depth study. Questions on topics assigned for independent study are included in the control measures.

COURSE POLICY

Policy on deadlines and retakes:
- Unexcused absences will be made up as scheduled by the teachers on duty.

-        Excused absences are made up on an individual schedule with the permission of the dean.

Policy on academic integrity:

It is obligatory to observe academic integrity by students, namely independent performance of all types of work, tasks, forms of control provided by the work program of this discipline:

-        references to sources of information in case of using ideas, developments, statements, information;

-        compliance with copyright and related rights legislation;

-        providing reliable information about the results of their own educational (scientific) activities, used research methods and sources of information.

Unacceptable in educational activities for participants in the educational process are:

-        the use of family or official ties to obtain a positive or higher grade during any form of control of learning outcomes or advantages in scientific work;

-        use of prohibited auxiliary materials or technical means (cribs, notes, micro-headphones, phones, smartphones, tablets, etc.) during control assessments;

-        passing the procedures for controlling the results of training by fictitious persons.

For violation of academic integrity, students may be brought to such academic responsibility:

-        lowering the results of the assessment of control work, assessment in the classroom, test, etc;

-        repeated passing of assessment (control work, test, etc.)

-        appointment of additional control assessments (additional individual tasks, control works, tests, etc.);

-        conducting an additional check of other works of the offender's authorship.

Policy on attendance and lateness:

Uniform: medical gown that completely covers the outer clothing, or medical pajamas, cap, mask, change of shoes.

Equipment: notebook, pen.

Health status: students with acute infectious diseases, including respiratory diseases, are not allowed to attend classes.

Lateness to classes is not allowed. A student who is late for the lesson may attend it, but if the teacher has put "ab" in the register, they must made it up in the general order.

Use of mobile devices:

The use of any mobile devices is prohibited. In case of violation of this paragraph, the student must leave the class and the teacher puts "ab" in the register, which they must made out up the general order.

Mobile devices can be used by students with the permission of the teacher if they are needed to complete the task.

Behaviour in the classroom:

The behavior of students and teachers in the classroom must be working and calm, strictly comply with the rules established by the Regulations on Academic Integrity and Ethics of Academic Relations at Odesa National Medical University, in accordance with the Code of Academic Ethics and Relations of the University Community of Odesa National Medical University, the Regulations on the Prevention and Detection of Academic Plagiarism in the Research and Educational Work of Higher Education Students, Researchers and Teachers of Odesa National Medical University.