Ministry of Education and Science of Ukraine
Ivan Franko National University of Lviv
Faculty of Biology
Department of Microbiology

"**APPROVE**"
Dean of the Faculty of Biology
_____ ass. prof. Khamar I. S.
_____"_____" 2018
(Resolved by the Scientific Council
of the Faculty of Biology
_____ "____", 2018,
minutes N ___)

«MOLECULAR MICROBIOLOGY»

PROGRAM
of the educational discipline

for the training of masters
Field of knowledge 09 «Biology»
Speciality 091 «Biology»
Language of study: Ukrainian

Lviv – 2018

DEVELOPED by: Ivan Franko National University of Lviv

DEVELOPER OF THE PROGRAM:
head of the Department of Microbiology Svitlana Hnatush

Program approved at the meeting of the Department of Microbiology

Minutes N 1 from August "28", 2018.

Head of the Department of Microbiology

_____/prof. Hnatush S. O./
(signature)

August "28", 2018.

Approved by the Methodical Council of the Faculty of Biology

Minutes N 1 from " ", 2018.

"_____"_____2018 Head _____/ass. prof. Honcharenko V. I./
(signature)

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INTRODUCTION

Program of the educational discipline "Molecular Microbiology" is developed according to the educational-professional program of the training of master by the speciality 091 Biology, educational-professional program «Microbiology».

The subject of educational discipline is molecular organization of genomes of prokaryotic and eukaryotic microorganisms and molecular processes in microorganisms' cells.

Interdisciplinary connections: Microbiology, Virology, Biochemistry, Genetics, Molecular Biology, Biotechnology.

Program of educational discipline consists of such subject modules:

1. Genome of microorganisms.
2. Genetic recombination in microorganisms.
3. Reactions of matrix synthesis. Mutagenesis and reparation.

1. Aim and tasks of educational discipline

1.1. Aim of educational discipline "Molecular Microbiology" is acquaintance of students with the molecular organization of genomes of prokaryotic and eukaryotic microorganisms, regulation of genes expression at the levels of transcription, translation and protein folding, and also replication, recombination and reparation of genetic material, processes of restriction and modification of DNA in microorganisms.

1.2. Main tasks of the discipline "Molecular Microbiology" are:

- to asquaint students with the last achievements of genomics of prokaryotic and eukaryotic microorganisms;
- to pay attention on the mechanisms of genes expression regulation in bacteria and yeasts, to consider levels of such regulation in details;
- to form knowledge about the methods of cloning the fragments of DNA, peculiarities of structure of vectors on the basis of prokaryotes and eukaryotes, creation of genomes libraries, restriction maps;
- to extend knowledge of students about the molecular mechanisms of replication, recombination and reparation of genetic material, processes of restriction and modification of DNA in prokaryotic and eukaryotic microorganisms.

1.3. According to the requirements of educational-professional program, students have to:

know:

- organization of genomes of microorganisms;
- processes of matrix synthesis in the cells of microorganisms;
- peculiarities of genetic recombination and reparation in prokaryotes;
- systems of restriction and modification in microorganisms;

be able to: select optimal experimental approaches for successful performance of task on the basis of acquainted knowledge and practical methods of molecular microbiology.

120 hours/4 ECTS credits are given for the study of educational discipline.

2. Information content of the educational discipline

Subject module 1. Genome of microorganisms

1. Introduction. Subject and tasks of Molecular Biology. History. Place of Molecular Biology in the system of Biological sciences. Modern methods of Molecular Biology.

2. Genome of prokaryotes. Development of conceptions about genetic apparatus of prokaryotes. Determination of terms: genome, bacterial chromosome, operone. Nucleoid of bacteria. Number of nucleoids of bacterial cells. Structure and chemical content of nucleiods. Number, shape and size of bacterial chromosomes. Interaction between DNA and proteins of bacteria. Histone-like proteins of bacteria. Domens of superspirilization of bacterial chromosomes. Role of RNA in the structure of nucleoid. Structure of linear chromosomes of bacteria. Interaction between chromosome and membrane of bacteria.

Number of genes in the genomes of different groups of bacteria and their size. Division of bacterial genes by their functions for categories. Peculiarities of genes set in archaea. Types of transcriptional units in bacteria. Introns in bacterial genes: introns of the 1^{st} and the 2^{nd} groups, pre-mRNA-like introns. Introns of archaea. Classification of structural genes of prokaryotes. Organization and principle of regulation of bacterial operons. Classical scheme of operon by Jacobe and Mono.

3. Genome of fungi. Generel review of organization of genomes of yeasts and moulds. Genes of chromosomes. Organization of mitochondrial genome of fungi. Mitochondrial genome of yeasts: size and nucleotide composition. Genes, which are coded by mitochondrial DNA of yeasts. Recombinations of mitochondrial genome of yeasts. Mitochondrial genome of *Neurospora*. Mitochondrial plasmids of *Neurospora*. Genomes of mitochondrial plasmids of *Neurospora*. Anomalies of mitochondrial genome and ageing. Transcription of mitochondrial genes and its regulation. Mitochondrial RNA-polymerase of *Saccharomyces*.

Regulation of transcription. RNA-processing: capping, polyadenilation and RNA-splicing. Ageing and destruction of RNA. Regulation of yeasts genome translation. Proteins of nuclear coding – regulators of mitochondrial translation.

Systems of *Saccharomyces cerevisiae* expression.

4. Plasmids. Determination of terms: plasmid, episome. Part of plasmid DNA in bacterial cells. Size of plasmid DNA. Forms of plasmids. Replication of plasmids. Mechanisms of circular plasmids replication (replication with the creation of "q-forms", replication by the mechanism of "substitution of chain" and by the mechanism of "rolling circle"). Mechanisms of replication of linear plasmids. Stages of replication of plasmids. Regulation of replication of plasmids. Coordination

between replication of bacterial chromosomes and plasmids. Number of plasmids copies. Segregation of plasmids during cell division. Incompatibility of plasmids. Instability. Interaction of plasmids with bacterial chromosomes. Mutability of bacteria, caused by plasmids integration into bacterial chromosomes and their excision. Recombination between plasmids. Features of bacteria, controlled by plasmids.

Conjugative plasmids. Structure of *E. coli* F-plasmid. Conjugative plasmids of gram-positive bacteria. Plasmids, coding the resistance of bacteria to antibacterial agents (R-plasmids). Identification of R-plasmids in bacterial populations. Plasmids, controlling synthesis of bacteriocines and toxins. Plasmids of biodegradation (D-plasmids). Their structure and distribution among bacteria. Sym-plasmids of nodules bacteria. Plasmids of lactobacteria. Ti- and Ri-plasmids of *Agrobacterium*. Mechanism of transference of Ti-plasmid T-DNA to plant cells. Role of plasmids in the evolution of prokaryotes.

5. Bacterial vector systems. Cloning plasmid vectors. Cosmids and phasmids. Their usage as molecular vector systems. Molecular vectors of bacteria of *Bacillus* genus. Usage of *Corynebacterium glutamicum* plasmids as the donors of genetic elements. Universal methods of plasmids insertion.

6. Mobile genetic elements of prokaryotes. IS-elements and transposons of bacteria. Molecular mechanisms of transposition. Replicative and non-replicative transposition. Regulation of transposition process. Changes of genome of microorganism, caused by transposing elements. Horizontal transfer of genes and its role in prokaryotes evolution.

Role of DNA restriction and modification systems. Methylation of DNA of phages and bacteria. Restriction of non-methylated DNA. Classification of restriction-modification systems. Enzymes of restriction and modification. Specificity of restrictases and methylases. Anti-restrictase mechanisms of bacteriophages.

Subject module 2. Genetic recombination in microorganisms

7. Genetic recombination in microorganisms. Conjugation. Conception and significance of genetic recombination in bacteria. Ways, leading to genetic recombination in bacteria. Heterotalicity of genetic exchange in bacteria. Formation of merozygotes in the processes of genetic information transfer in bacteria. Types of genetic recombination. General recombination (homological recombination). Site-specific recombination. Formation of heteroduplex region. Gene conversion. Enzymology of heteroduplex region. RecA protein and its role in the homological recombination. Role of nucleases Rec B, C and multi-enzyme complex RecBCD in the realization of homological recombination. Structure of intasome.

Distribution of conjugation among bacteria. Study of the nature of *E. coli* factor of fertility. DNA F-factor. Hfr-donors. Interaction of F-factor with chromosome of *E. coli*. Sites of integration of F-factor into *E. coli* chromosome.Excision of F-factor. F'-factors. Primary and secondary F'-donors. Stability of F+- and F'-donors. Study of dynamics of chromosome markers transfer in the process of conjugation. Role of conjugation in the evolution of bacteria.

Principles of construction of genetic maps of bacteria: method of interrupted conjugation, conjugative mapping by the frequency of recombination, transductive mapping.

8. Genetic transformation in bacteria. Discovery of genetic transformation in bacteria. Distribution of natural transformation among bacteria. Role of genetic transformation in the horizontal transfer of genes. *Bacillus subtilis, Streptococcus pneumoniae, Haemophilus influenzae* as the model objects for the study of genetic transformation. Characteristics of competence state in bacteria. Genes, controlling competence of Gram-positive bacteria (com-genes). Peptides – factors of competence of Gram-positive bacteria. Peculiarities of the competence state in Gram-negative bacteria. Transformosomes of hemophilic bacteria. Frequency of transformants appearance. Analysis of coupling of chromosomal genes using transformation. Genetic maps of bacteria, built using transformation. Transformation by plasmid DNA. Differences between mechanisms of transformation by plasmid and chromosome DNA. Genetic transfection of bacteria by phage DNA. Genetic transformation and transfection as one of the main stages of genetic engineering experiment. Artificial methods of insertion of DNA into bacterial cells. Transformation by plasmid and transfection by phage DNA of bacterial protoplasts.

9. Transduction. Discovery of transduction. Scheme of transduction experiment. Types of transduction: specific, non-specific (general), abortive. Formation of phage particles, performing specific transduction. Connection of specific transduction with the lysogenic state of bacteria. Frequency of transductants appearance. Obtaining of Ltf- and Htf-lysates. Defectivity of transducing phages. Partial heterozygocity of transductants. Formation of phage particles, performing non-specific transduction. Recombination between transducing DNA and DNA of recipient cell. Capsduction. Transduction of plasmid DNA. Usage of transduction in the genetic construction. Role of transduction in the mutability and evolution of bacteria.

Subject module 3. Reactions of matrix synthesis. Mutagenesis and reparation

10. Reduplication of DNA in prokaryotes. The half-conservative mechanism of reduplication of DNA. Conseptions of replicon and replisome. Replication "fork". Types of replication. Mechanisms of biosynthesis of DNA. Role of matrix, dNTPs, formation of complementary product. Structure of primosome and process of its formation. Role of DNA-polymerase 3 in the replication. Mechanisms of copying of lagging strand. DNA-ligases.

11. Transcription in prokaryotes. Promotors and terminators. Transcriptone. DNA-dependent RNA polymerases. Cycle of DNA-dependent transcription. Processing of the primary transcripts. Main ways of the regulation of transcription. Regulation of transcription at the level of initiation: proteins-activators, proteins-repressors, sigma-factor.

12. Translation in prokaryotes. Molecular organization of prokaryotic ribosomes. Informative RNA as a matrix for protein synthesis. Mechanism of translation. Stages of protein biosynthesis: initiation, elongation and termination of translation. Peculiarities of translation in prokaryotes. Genetic code.

13. Posttranslational control and modification of proteins. Posttranslational modification of proteins in bacteria: phosphorylation, S-thiolation, hydroxylation, N-glycosylation, etc. Non-covalent modification of enzymatic activity and covalent processing of proteins. Allosteric regulation of activity of enzymes. Intracellular compartmentalization of enzymes and other proteins in prokaryotes.

14. United metabolic networks and pathways of signals transfer in prokaryotes. Metabolic pathways and their regulation. Modeling of metabolic networks. Databases of metabolic pathways and networks: Kyoto Encyclopedia of Genes and Genomes (KEGG), EcoCyc, BioCyc and metaTIGER. Operons. Modulons. Methods of studying the general regulative networks. Sensor systems of reception.

15. Inheritable and non-inheritable forms of mutability in prokaryotes. R-S-dissociations of bacteria. Classification of mutations in prokaryotes and mechanisms of their emergence. Genomic and gene mutations. Mutations, emerging in the process of DNA replication. Induced and spontaneous mutagenesis. Genes-mutators. Classification of mutagens of chemical origin (analogs of bases, alkilating agents, nitrous acid, acridine stains). Classification of physical mutagenic factors (UV-rays, radiation, electromagnetic radiation). Mechanism of action of chemical mutagens on prokaryotes cells. Types of DNA damages, emerging under the syfluence of chemical and physical mutagens.

16. Reparation in prokaryotes. Types of reparative systems of prokaryotes. Main mechanisms of reparative systems action. Light reparation. Excision reparation. Reparation of non-paired bases. SOS-response. System of induced reparation. Role of reparation enzymes: N-glycosylases, apurine endonuclease, enzymes of recombination complex, DNA-polymerase 1, DNA-ligase in the process of reparation of damaged DNA. Molecular process of their functioning, connection with mutation process.

3. Recommended literature

1. Alberts V. Molecular biology of cell. – Moscow: Mir, 2000. – 512 p. [in Russian]
2. Mushkambarov N. N., Kuznietsov S. L. Molecular biology. – Moscow: Medical Infromation Agency, 2003. – 287 p. [in Russian]
3. Patrushev L. I. Expression of genes. Moscow: Nauka, 2000. – 527 p. [in Russian]
4. Senger M., Berg P. Genes and genomes. – Moscow: Mir, 1998. V. 1–2. – 391 p. [in Russian]

5. Syvolob A. V. Molecular biology. – Kyiv: editorial-polygraphic center "Kyiv University", 2008. – 384 p. [in Ukrainian]
6. Modern microbiology: prokaryotes: in 2 vol. / ed. by Y. Lengeler, H. Drevs, H. Shlegel. – Moscow: Mir, 2005. – Vol. 1. – 656 p., Vol. 2. – 496 p. [in Russian]
7. Stoliar O. B. Molecular biology. – Ternopil: Handbooks and Manuals, 2014. – 224 p. [in Ukrainian]
8. Fedorenko V. O., Ostash B. O., Honchar M. V., Rebets Y. V. Large practice in genetics, genetic engineering and analytical biotechnology of microorganisms. – Lviv: Editorial Center of Ivan Franko LNU, 2007. – 279 p. [in Ukrainian]
9. The complete genome sequence of *Esherichia coli* K12 / Blattner F.R., Plunkett G., Bloch C.A., Perna N.T., Burland V. [et al.] // Science. – 1997. – Vol. 277. – P. 1453–1462.
10. *Walker G.M.* Yeast Physiology and Biotechnology.– Chichester, N.Y: John Wiley & Sons, 1998. – 320 p.
11. *Streips U., Yasbin R.* Modern microbial genetics. Second Edition. – Wiley-Liss, Inc., 2002. – 655 p.
12. Genomic sequence diversity and population structure of *Saccharomyces cerevisiae* assessed by RAD-seq / Cromie G., Hyma K., Ludlow C. [et al.] // G3 (Bethesda). – 2013. – Vol. 3, № 12. – P. 2163–2171. doi: 10.1534/g3.113.007492.
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15. Translation in prokaryotes / Rodnina M. // Cold Spring Harb. Perspect. Biol. – 2018. doi: 10.1101/cshperspect.a032664/
16. DNA damage responses in prokaryotes: regulating gene expression, modulating growth patterns, and manipulating replication forks / Kreuzer K. // Cold Spring Harb. Perspect. Biol. – 2013. – Vol 5: a012674. – P. 1–23. doi: 10.1101/cshperspect.a012674.
17. Sensor domains of two-component regulatory systems / Cheung J., Hendrickson W. // Curr. Opin. Microbiol. – 2010. – Vol. 13, № 2. – P. 116–123. doi:10.1016/j.mib.2010.01.016.
18. Bacterial histidine kinase as signal sensor and transducer /. Khorchid A, Ikura M. // Int. J. Biochem. Cell Biol. – 2005. – BC-2058. – P. 1 – 6.
19. http://mfa.od.ua/index.htm

4. Form of the final control of the success of education

Final control – exam.

5. Methods of control

Control of students' knowledge is performed by the 100-point scale.

The highest number of points during the control of students' skills on the discipline with exam is 50 points for the current progress and 50 points during the exam.

Scale: of the institution, national and ECTS

ECTS	Points	National scale	
A	90 – 100	5	Excellent
B	81-89	4	Good
C	71-80		
D	61-70	3	Satisfactory
E	51-60		
FX	21–50		Not satisfactory
F	0–20		

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