APPROVED
Head of Landau Phystech-School of
Physics & Research
**A.V. Rogachev**

**Work program of the course (training module)**

**course:** Modern Methods of Genetic Engineering, Genome Editing, Metabolic Engineering/Современные методы генетической инженерии, геномное редактирование, метаболическая инженерия

**major:** Applied Mathematics and Physics

**specialization:** General and Applied Physics/Общая и прикладная физика
Landau Phystech-School of Physics & Research
Chair of Biophysics

**term:** 1

**qualification:** Master

Semester, form of interim assessment: 1 (fall) - Exam

Academic hours: 30 AH  in total, including:
    lectures: 30 AH.
    seminars: 0 AH.
    laboratory practical: 0 AH.

Independent work: 30 AH.

Exam preparation: 30 AH.

In total:  90 AH, credits in total: 2

Number of course papers, tasks: 2

Authors of the program:

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    S.V. Bazhenov, candidate of biological sciences
    S.V. Mashko

The program was discussed at the Chair of Biophysics 07.04.2023

**Annotation**

Genetic engineering is the methodological basis of all branches of modern genetic engineering and biotechnology, which are currently successfully used to create artificial organisms and living systems that produce many practically useful chemical compounds and macromolecules used, for example, in industry and medicine. It is a synthetic discipline that is a methodological application of molecular genetics. The current course will present the most relevant methods for constructing recombinant DNA, starting with polymerase chain reaction and modification of bacterial plasmids used as vectors, with examples of commercially available enzymes used for this purpose in laboratories, and ending with modern approaches to editing the genome of pro- and eukaryotic cells based on recombination and CRISPR/Cas methodology. Genetic engineering makes it possible to carry out metabolic engineering - a directed change in metabolism to create artificial producers of practically important biological compounds and molecules for their industrial production.

## 1. Study objective

**Purpose of the course**

> formation and improvement of students' competencies in the field of genetic engineering, familiarization with modern approaches to genome editing and metabolic engineering

**Tasks of the course**

> 1) Acquaintance of students with the general principles and modern methods of constructing recombinant DNA.
> 2) Training in modern approaches to editing the genome of pro- and eukaryotic cells based on directed homologous recombination.
> 3) Introduction to metabolic engineering, training in approaches to the design of producers of cellular metabolites, and to the development of technologies for the biosynthesis of metabolites for modern production.

## 2. List of the planned results of the course (training module), correlated with the planned results of the mastering the educational program

Mastering the discipline is aimed at the formation of the following competencies:

| Code and the name of the competence | Competency indicators |
|---|---|
| UC-1 Use a systematic approach to critically analyze a problem,and develop an action plan | UC-1.1 Systematically analyze the problem situation, identify its components and the relations between them |
| | UC-1.2 Search for solutions by using available sources |
| | UC-1.3 Develop a step-by-step strategy for achieving a goal, foresee the result of each step, evaluate the overall impact on the planned activity and its participants |
| UC-4 Use modern communication tools in the academic and professional fields, including those in a foreign language | UC-4.1 Exchange business information in oral and written forms in Russian and at least one foreign language |
| | UC-4.3 Present the results of academic and professional activities at various academic events, including international conferences |
| | UC-4.4 Use modern ICT tools for academic and professional collaboration |
| Gen.Pro.C-1 Gain fundamental scientific knowledge in the field of physical and mathematical sciences | Gen.Pro.C-1.1 Apply fundamental scientific knowledge in the field of physical and mathematical sciences |
| | Gen.Pro.C-1.2 Consolidate and critically assess professional experience and research findings |
| Gen.Pro.C-2 Acquire an understanding of current scientific and technological challenges in professional settings, and scientifically formulate professional objectives | Gen.Pro.C-2.1 Assess the current state of mathematical research within professional settings |
| | Gen.Pro.C-2.2 Assess the relevance and practical importance of research in professional settings |
| | Gen.Pro.C-2.3 Understand professional terminology used in modern scientific and technical literature and present scientific results in oral and written form within professional communication |

| | |
|---|---|
| Gen.Pro.C-3 Select and/or develop approaches to professional problem-solving with consideration to the limitations and specifics of different solution methods | Gen.Pro.C-3.1 Analyze problems, plan research strategy to achieve solution(s), propose, and combine solution approaches |
| | Gen.Pro.C-3.2 Employ research methods to solve new problems and apply knowledge from various fields of science (technology) |
| | Gen.Pro.C-3.3 Gain knowledge of analytical and computational methods of problem-solving, understand the limitations of the implementation of the obtained solutions in practice |
| Gen.Pro.C-4 Successfully perform a task, analyze the results, and present conclusions, apply knowledge and skills in the field of physical and mathematical sciences and ICTs | Gen.Pro.C-4.1 Apply ICT knowledge and skills to find and study scientific literature and use software products |
| | Gen.Pro.C-4.2 Apply knowledge in the field of physical and mathematical sciences to solve problems, make conclusions, and evaluate the obtained results |
| | Gen.Pro.C-4.3 Justify the chosen method of scientific research |
| Pro.C-1 Assign, formalize, and solve tasks, develop and research mathematical models of the studied phenomena and processes, systematically analyze scientific problems and obtain new scientific results | Pro.C-1.1 Locate, analyze, and summarize information on current research findings within the subject area |
| | Pro.C-1.3 Apply theoretical and/or experimental research methods to a specific scientific task and interpret the obtained results |
| Pro.C-3 Use research and testing equipment (devices and installations, specialized software) in a selected subject field | Pro.C-3.1 Understand the operating principles of the equipment and specialized software |
| | Pro.C-3.2 Conduct an experiment (simulation) using research equipment (software) |
| | Pro.C-3.3 Evaluate the accuracy of the experimental (numerical) results |

## 3. List of the planned results of the course (training module)

As a result of studying the course the student should:

know:

- Basic principles and methods of constructing recombinant DNA.
- Modern approaches to genome editing based on recombination and CRISPR / Cas methodology.

be able to:

- Apply methods of constructing recombinant DNA to solve fundamental professional problems.
- Creatively use in scientific activity knowledge about genome editing based on recombination and CRISPR / Cas methodology.
- Highlight and systematize the main ideas in scientific texts.
- Critically evaluate any incoming information, regardless of the source.
- Generate new ideas and methodological solutions.
- Carry out the design of their scientific activities.
- Present your scientific results in oral reports.

master:

- Methods of theoretical and experimental research.
- Skills of search (including using information systems and databases), processing, analysis and systematization of information.
- Skills of critical analysis and assessment of modern scientific achievements.

## 4. Content of the course (training module), structured by topics (sections), indicating the number of allocated academic hours and types of training sessions

4.1. The sections of the course (training module) and the complexity of the types of training sessions

| | | Types of training sessions, including independent work |
|---|---|---|
| | | |

| № | Topic (section) of the course | Lectures | Seminars | Laboratory practical | Independent work |
|---|---|---|---|---|---|
| 1 | Introduction | 2 | | | 2 |
| 2 | Assembly of hybrid DNA | 2 | | | 2 |
| 3 | Construction of hybrid DNA molecules. Mutagenesis | 2 | | | 2 |
| 4 | Genomic editing in prokaryotic cells | 2 | | | 2 |
| 5 | Genomic editing in eukaryotic cells | 2 | | | 2 |
| 6 | DNA sequencing | 2 | | | 2 |
| 7 | Metabolic engineering (ME) | 2 | | | 2 |
| 8 | ME development stages. Precision directed ME | 2 | | | 2 |
| 9 | Systems ME | 2 | | | 2 |
| 10 | Genomics, trancriptomics and proteomics for metabolomics | 2 | | | 2 |
| 11 | Fluxomics | 2 | | | 2 |
| 12 | Application of 13C-MFA for medical devices | 2 | | | 2 |
| 13 | Induction and dynamic control | 2 | | | 2 |
| 14 | ME successes | 2 | | | 2 |
| 15 | Student reports, consultation before the exam | 2 | | | 2 |
| AH in total | | 30 | | | 30 |
| Exam preparation | | 30 AH. | | | |
| Total complexity | | 90 AH., credits in total 2 | | | |

4.2.   Content of the course (training module), structured by topics (sections)

Semester: 1 (Fall)

1. Introduction

The subject of genetic engineering. Nucleic acids (NA). Central dogma of molecular biology. The main types of enzymes used in genetic engineering. The history of the development of genetic engineering. Cloning basics. Cloning of genes. Markers of positive and negative selection.

2. Assembly of hybrid DNA

Assembly of hybrid DNA in vitro using individual enzymes and their mixtures (e.g. In-fusion, Gibson Assembly, GoldenGate). The concept of BioBricks. Hybrid DNA assembly methods using homologous recombination in cells.

3. Construction of hybrid DNA molecules. Mutagenesis

Introduction of point mutations into plasmid constructs using PCR. Synthesis of DNA sequences de novo. Spontaneous and induced mutagenesis. Mutagens. Mutagenesis and selection. Transposon mutagenesis.

4. Genomic editing in prokaryotic cells

Bacterial genome editing using homologous recombination. Lambda red recombination to increase the efficiency of enterobacteria genome editing. Use of positive selection markers for genomic editing. Point editing of the bacterial chromosome. Homologous recombination with DNA delivery within the pKnock mobilizable suicide vector. Transduction.

## 5. Genomic editing in eukaryotic cells

Mutagenesis in eukaryotic cells. CRISPR-Cas9, TALENs, ZFN and meganucleases - nucleases for genetic engineering of eukaryotes.

## 6. DNA sequencing

DNA sequencing technologies:
1st generation (Sanger method);
2nd generation (Illumina, Ion Torrent, 454GS FLX and SOLiD 5500xl);
3rd generation (PacBio, Oxford Nanopore).

## 7. Metabolic engineering (ME)

Metabolic engineering as a tool for obtaining microbes with desired properties.

## 8. ME development stages. Precision directed ME

Three stages of ME development (brief description and distinctive features, examples of achievements at individual stages).

## 9. Systems ME

Systems ME (brief characteristics of the components, fundamental difference from precision-directed ME, cyclicity of research).

## 10. Genomics, trancriptomics and proteomics for metabolomics

X-omics technologies: Genomics, Transcriptomics, Proteomics, Metabolomics - the essence of "post-genomic" methods, the results obtained, successful examples of use for solving ME problems.

## 11. Fluxomics

Why Fluxomics is often called the quintessence of all other X-omics technologies. FBA and 13C-MFA - the commonality of the mathematical apparatus of the quasi-stationary solution of stoichiometric equations and the difference in the use of models, approximations and experimental data, and therefore the results.

## 12. Application of 13C-MFA for medical devices

13C-MFA principles and results obtained for developing an experimental strategy and analyzing the results of ME.

## 13. Induction and dynamic control

Regulated cultivation - similarities and differences between inducible expression and "Dynamic metabolic control".

## 14. ME successes

By the 30th anniversary of ME in 2021 - examples of outstanding success (creation of producers of aa, 1,3-propanediol, 7-ADCA, 1,4-butanediol, arteminisin, isobutanol).

15. Student reports, consultation before the exam

Student reports based on scientific publications on the latest achievements in genetic engineering. Advice on course materials before the exam.

## 5. Description of the material and technical facilities that are necessary for the implementation of the educational process of the course (training module)

1. Classroom with a media projector and screen, Internet access.
2. Required software.
3. Providing independent work - databases on logs.

## 6. List of the main and additional literature, that is necessary for the course (training module) mastering

Main literature

1. Molecular Cloning: A Laboratory Manual (Third Edition). Sambrook J., Russell D.W. Cold Spring Harbor Laboratory Press. 2000.
2. Lee S. Y., Lee D. Y., Kim T. Y. Systems biotechnology for strain improvement //Trends in biotechnology. – 2005. – Т. 23. – №. 7. – С. 349-358.
3. Goldberg I., Rokem J. S., Pines O. Organic acids: old metabolites, new themes //Journal of Chemical Technology and Biotechnology. – 2006. – Т. 81. – №. 10. – С. 1601-1611.
4. Magnuson J. K., Lasure L. L. Organic acid production by filamentous fungi //Advances in fungal biotechnology for industry, agriculture, and medicine. – Springer US, 2004. – С. 307-340.
5. Leuchtenberger W., Huthmacher K., Drauz K. Biotechnological production of amino acids and derivatives: current status and prospects //Applied Microbiology and Biotechnology. – 2005. – Т. 69. – №. 1. – С. 1-8.
6. Esaki N. et al. Enzymology of amino acid production //Biotechnology Set, Second Edition. – 1996. – С. 503-560.

Additional literature

1. Молекулярная биология. Рибосомы и биосинтез белка, Электронная версия печатной публикации / А. С. Спирин. — Москва, Лаборатория знаний, 2019
2. Генетика с основами селекции, учебник для студентов высших учебных заведений /С. Г. Инге-Вечтомов. Санкт-Петербург, Изд-во Н-Л, 2015

3. Льюин Б., «Гены», изд. Бином: Лаборатория знаний, 2012.

4. Brakmann S., Johnsson K. Directed molecular evolution of proteins. – Wiley-VCH, 2002.

5. Georgiou G. Directed evolution library creation. – Totowa, New Jersey : Humana Press, 2003.

6. de Winde H. Functional genetics of industrial yeasts; of ancient skills and modern applications. – Springer Berlin Heidelberg, 2003. – C. 1-16.

7. Reymond J. L., Babiak P. Screening systems //White biotechnology. – Springer Berlin Heidelberg, 2007. – C. 31-58.

8. Hilterhaus L., Liese A. Building blocks //White biotechnology. – Springer Berlin Heidelberg, 2007. – C. 133-173.

9. Kamm B., Kamm M. Biorefineries–multi product processes //White Biotechnology. – Springer Berlin Heidelberg, 2007. – C. 175-204.

10. Kuchner O., Arnold F. H. Directed evolution of enzyme catalysts //Trends in biotechnology. – 1997. – Т. 15. – №. 12. – C. 523-530.

11. Завильгельский Г.Б., Манухов И.В. Генетическая инженерия. – М.: изд. МФТИ, 2012.

12. Ishino, S.; Ishino, Y. DNA polymerases as useful reagents for biotechnology - The history of developmental research in the field. Front. Microbiol. 2014, 5, 465, doi:10.3389/FMICB.2014.00465.

13. Martín-Alonso, S.; Frutos-Beltrán, E.; Menéndez-Arias, L. Reverse Transcriptase: From Transcriptomics to Genome Editing. Trends Biotechnol. 2021, 39, 194–210, doi:10.1016/j.tibtech.2020.06.008.

14. Völler, J.S. Enhancing DNA sequencing. Nat. Catal. 2018 17 2018, 1, 481–481, doi:10.1038/s41929-018-0120-7.

15. Rosano, G.L.; Ceccarelli, E.A. Recombinant protein expression in Escherichia coli: Advances and challenges. Front. Microbiol. 2014, 5, 172, doi:10.3389/FMICB.2014.00172.

16. Gavrilov, M.; Yang, J.Y.C.; Zou, R.S.; Ma, W.; Lee, C.Y.; Mohapatra, S.; Kang, J.; Liao, T.W.; Myong, S.; Ha, T. Engineered helicase replaces thermocycler in DNA amplification while retaining desired PCR characteristics. Nat. Commun. 2022 131 2022, 13, 1–14, doi:10.1038/s41467-022-34076-0.

17. Hughes, R.A.; Ellington, A.D. Synthetic DNA Synthesis and Assembly: Putting the Synthetic in Synthetic Biology. Cold Spring Harb. Perspect. Biol. 2017, 9, doi:10.1101/CSHPERSPECT.A023812.

18. Cain, A.K.; Barquist, L.; Goodman, A.L.; Paulsen, I.T.; Parkhill, J.; van Opijnen, T. A decade of advances in transposon-insertion sequencing. Nat. Rev. Genet. 2020 219 2020, 21, 526–540, doi:10.1038/s41576-020-0244-x.

## 7. List of web resources that are necessary for the course (training module) mastering

NCBI, molbiol.ru, PDB, swissprot.

## 8. List of information technologies used for implementation of the educational process, including a list of software and information reference systems (if necessary)

When preparing and conducting lectures, the Internet is used.

In addition, Libre Office is used, as well as the Ink Scape graphics package.

## 9. Guidelines for students to master the course

A student studying the discipline must, on the one hand, master the general conceptual apparatus, and on the other hand, must learn to apply theoretical knowledge in practice.

As a result of studying the discipline, the student must know the basic definitions and concepts, be able to apply the knowledge gained to solve various problems.

Successful completion of the course requires:

- attendance of all classes provided for by the curriculum for the discipline;

- keeping a synopsis of classes;

- student's intense independent work.

Independent work includes:

- reading recommended literature;

- study of educational material, preparation of answers to questions intended for independent study;

- solving problems offered to students in the classroom;

- preparation for the performance of tasks of the intermediate certification.

An indicator of mastery of the material is the ability to answer questions on the topics of the discipline without a synopsis.

It is important to achieve an understanding of the material being studied, not its mechanical memorization. If a student finds it difficult to study certain topics, questions, he/she should seek advice from a teacher.

Intermediate control of students' knowledge is possible in the form of solving problems in accordance with the topic of classes.

**Assessment funds for course (training module)**

| | |
|---|---|
| **major:** | Applied Mathematics and Physics |
| **specialization:** | General and Applied Physics/Общая и прикладная физика |
| | Landau Phystech-School of Physics & Research |
| | Chair of Biophysics |
| **term:** | 1 |
| **qualification:** | Master |

Semester, form of interim assessment: 1 (fall) - Exam

**Authors:**

I.V. Manukhov, doctor of biological sciences
S.V. Bazhenov, candidate of biological sciences
S.V. Mashko

# 1. Competencies formed during the process of studying the course

| Code and the name of the competence | Competency indicators |
|---|---|
| UC-1 Use a systematic approach to critically analyze a problem,and develop an action plan | UC-1.1 Systematically analyze the problem situation, identify its components and the relations between them |
| | UC-1.2 Search for solutions by using available sources |
| | UC-1.3 Develop a step-by-step strategy for achieving a goal, foresee the result of each step, evaluate the overall impact on the planned activity and its participants |
| UC-4 Use modern communication tools in the academic and professional fields, including those in a foreign language | UC-4.1 Exchange business information in oral and written forms in Russian and at least one foreign language |
| | UC-4.3 Present the results of academic and professional activities at various academic events, including international conferences |
| | UC-4.4 Use modern ICT tools for academic and professional collaboration |
| Gen.Pro.C-1 Gain fundamental scientific knowledge in the field of physical and mathematical sciences | Gen.Pro.C-1.1 Apply fundamental scientific knowledge in the field of physical and mathematical sciences |
| | Gen.Pro.C-1.2 Consolidate and critically assess professional experience and research findings |
| Gen.Pro.C-2 Acquire an understanding of current scientific and technological challenges in professional settings, and scientifically formulate professional objectives | Gen.Pro.C-2.1 Assess the current state of mathematical research within professional settings |
| | Gen.Pro.C-2.2 Assess the relevance and practical importance of research in professional settings |
| | Gen.Pro.C-2.3 Understand professional terminology used in modern scientific and technical literature and present scientific results in oral and written form within professional communication |
| Gen.Pro.C-3 Select and/or develop approaches to professional problem-solving with consideration to the limitations and specifics of different solution methods | Gen.Pro.C-3.1 Analyze problems, plan research strategy to achieve solution(s), propose, and combine solution approaches |
| | Gen.Pro.C-3.2 Employ research methods to solve new problems and apply knowledge from various fields of science (technology) |
| | Gen.Pro.C-3.3 Gain knowledge of analytical and computational methods of problem-solving, understand the limitations of the implementation of the obtained solutions in practice |
| Gen.Pro.C-4 Successfully perform a task, analyze the results, and present conclusions, apply knowledge and skills in the field of physical and mathematical sciences and ICTs | Gen.Pro.C-4.1 Apply ICT knowledge and skills to find and study scientific literature and use software products |
| | Gen.Pro.C-4.2 Apply knowledge in the field of physical and mathematical sciences to solve problems, make conclusions, and evaluate the obtained results |
| | Gen.Pro.C-4.3 Justify the chosen method of scientific research |
| Pro.C-1 Assign, formalize, and solve tasks, develop and research mathematical models of the studied phenomena and processes, systematically analyze scientific problems and obtain new scientific results | Pro.C-1.1 Locate, analyze, and summarize information on current research findings within the subject area |
| | Pro.C-1.3 Apply theoretical and/or experimental research methods to a specific scientific task and interpret the obtained results |
| Pro.C-3 Use research and testing equipment (devices and installations, specialized software) in a selected subject field | Pro.C-3.1 Understand the operating principles of the equipment and specialized software |
| | Pro.C-3.2 Conduct an experiment (simulation) using research equipment (software) |
| | Pro.C-3.3 Evaluate the accuracy of the experimental (numerical) results |

## 2. Competency assessment indicators

As a result of studying the course the student should:

**know:**
- Basic principles and methods of constructing recombinant DNA.
- Modern approaches to genome editing based on recombination and CRISPR / Cas methodology.

**be able to:**
- Apply methods of constructing recombinant DNA to solve fundamental professional problems.
- Creatively use in scientific activity knowledge about genome editing based on recombination and CRISPR / Cas methodology.
- Highlight and systematize the main ideas in scientific texts.
- Critically evaluate any incoming information, regardless of the source.
- Generate new ideas and methodological solutions.
- Carry out the design of their scientific activities.
- Present your scientific results in oral reports.

**master:**
- Methods of theoretical and experimental research.
- Skills of search (including using information systems and databases), processing, analysis and systematization of information.
- Skills of critical analysis and assessment of modern scientific achievements.

## 3. List of typical control tasks used to evaluate knowledge and skills

Examples of topics of individual courseworks/reports:

1. Obtaining mutations using the CRISPR/Cas9 system
2. Epistemological roots and structural components of bioengineering
3. Metabolic Engineering - the birth and evolution of the term, modern definition
4. By the 30th anniversary of ME in 2021 - examples of outstanding successes (creation of producers of aa, 1,3-propanediol, 7-ADCA, 1,4-butanediol, arteminisin, isobutanol)
5. Advances in genetic selection of amino acid producers

## 4. Evaluation criteria

Checking questions:
1. The subject of genetic engineering, the central dogma of molecular biology
2. Construction of hybrid plasmids, general principles with examples of specific methods.
3. DNA homologous recombination, genomic editing methods using homologous recombination.
4. DNA sequencing. Areas of applicability of 1st, 2nd and 3rd generation sequencing methods.
5. Chimeric proteins, differences in protein aggregation and oligomerization.
6. DNA sequencing
7. Metabolic engineering (ME)
8. ME development stages. Precision directed ME
9. Systems ME
10. Genomics, trancriptomics and proteomics for metabolomics
11. Fluxomics

Exam question papers:

1
The subject of genetic engineering.
Enzymes used in genetic engineering.
Restriction-modification systems.
RNA polymerase. DNA polymerase.
Alkaline phosphatase and polynucleotide kinase

Nucleases
Topoisomerase
DNA ligase

## 2

Replication, transcription, translation: the main differences in pro- and eukaryotes.
Autonomous units of replication (plasmids, bacteriophages, chromosomes). Replicons.
Incompatibility of plasmids. Mobilization of plasmids.
Markers used for selection.

## 3

Isolation of plasmid DNA. Isolation of total DNA. Isolation of RNA.
Separation of DNA fragments by electrophoresis in agarose and polyacrylamide gels.
Principle of amplification of DNA fragments using PCR.
Cloning of fragments obtained by PCR. Cloning into a T-vector.
Spontaneous mutagenesis. The main types of DNA damage.
DNA repair systems in bacteria on the example of E. coli.

## 4

Homologous DNA recombination.
Site-directed mutagenesis.
Obtaining mutations in the E. coli chromosome. Selection markers.

## 5

The concept of BioBricks. Hybrid DNA assembly methods: In-fusion, LIC, TEDA, TPA, EFC, PIPE, MOE-PCR, Hot fusion, NEBuilder, DATEL, CPEC, SLIC, SLiCE, USER, Gibson Assembly, homologous recombination in cells.

## 6

Obtaining mutations using the CRISPR/Cas9 system.
Translation in bacteria and eukaryotes.
Post-translational level of regulation of protein activity.
Transfection

## 7

Epistemological roots and structural components of bioengineering. The relationship between the individual components.
What are the specific features of 13C-MFA in the case of parallel experiments (PLE instead of SLE), differing in the labeling of the initial substrate?

## 8

Metabolic Engineering - the birth and evolution of the term, modern definition; fundamental direction of research and their practical significance. Development stages, their essence, methodological basis and fundamental differences.
What are the prospects for analyzing the "kinetics" of changes in fluxes during the cultivation of microorganisms? Successful examples of 13C-MFA use in modern metabolic engineering.

## 9

By the 30th anniversary of ME in 2021 - examples of outstanding successes (creation of producers of aa, 1,3-propanediol, 7-ADCA, 1,4-butanediol, arteminisin, isobutanol).
Why Fluxomics is often considered the "top" of all modern X-omics technology. What is the result of the calculation of modern Fitting programs in 13C-MFA, what gives the calculation of the design of the experiment, confidence intervals for the values of flows?

## 10

Amino acids are a traditional biotechnology product and a target for ME. Advances in genetic selection of amino acid producers. Difficulties and main modern ways of creating strains-producers of aa.

Analysis of the statistics of solving the problem of flows. Principles of using the Monte Carlo method to establish confidence intervals with a cutoff of a certain number of quantiles to determine the 95% or 68% confidence level.

## 11

What is the fundamental difference in the early and modern use of the methods of mutagenesis and selection. Concepts of Inverse / Inverted MI and Reverse Genetics.

Experiment in 13C-MFA: NMR and GC-MS, LC-MS / MS. Possibilities and prospects of using for solving stationary problems based on the analysis of AA and metabolites.

## 12

Modern methods of mutagenesis (MAGE, TRMR, Global transcriptional engineering, etc.). The need for development and the importance of HT (high-throughput) -based approaches for modern MI.

Theoretical approaches to the experimental determination of flux values characterizing the proposed model, including the stoichiometry of all reactions and permutations of carbon atoms. Isotopomer concept.

## 13

What is the fundamental and methodological difference in the approaches used to create producers of 1,3-propanediol and 1,4-butanediol.

Methods for modeling the distribution of 13C carbon atoms from molecules of the initial substrate into intracellular metabolites and proteinogenic AAs (AMM, IMM, cumomers, EMU). Available computer software (13CFLUX, Metran, OpenFLUX, OpenFLUX2).

## 14

What are the possibilities of creating various types of biofuels using microbial producers based on?

Basic principles of stationary 13C-MFA. Stoichiometric models of metabolism from "cow" to "full-genomic". General scheme of the 13C-MFA experiment. Experimental and theoretical stages. Stationary approximation of 13C-MFA - the possibility of using "proteinogenic" amino acids to obtain information about metabolic fluxes.

## 15

What is the "evolution of proteins" and how to carry out such experiments.

Two fundamentally different directions of research of modern Fluxomics - FBA and 13C-MFA - conditionally, "theoretical" and "experimental" approaches. General principles. Theoretical undecidability of reversible, cyclic, etc. reactions within the FBA.

## 16

What factors underlie the decision to create (modernize) a producer strain of a new or already known product by ME methods with the aim of using it in a new (or already existing) industrial production.

Systems biology - definition of the concept and research methods. Examples of the use of methods of systems biology in modern ME (at least one example of research on ME, in which the basis of the strategy was based on information obtained from one of the X-omics technologies).

## 17

What are the most important scientific, methodological and organizational approaches were developed and tested when creating producers of artemisinic acid and then artemisinin, and what was the charitable initiative of the authors of the drug.

Analysis of metabolites. Types of modern "metabolic" analysis. The fundamental difficulty of obtaining reliable results. Devices and methods used for the analysis of metabolites; main problems and achievements of Metabolomics. The importance of sample preparation.

## 18

What is the principal strategy for conducting ME experiments. Give examples of the need for this type of strategy.

Study of metabolic enzymes. Advantages of using the "Proteomics" methods in modern studies of the structure and function of proteins.

19

Modern methods of editing genomes of microorganisms. From plasmid modifications to "randomization" of target sequences in the chromosome based on Recombineering with contra-selection via SacB, I-SceI, CRISPR / Cas. Combined strategies (e.g., Dual In/Out).

Transcriptomics - the role in the system of X-ohm research in systems biology, the essence of the method and the evolution of experimental approaches. Examples of detecting genes of "new enzymes". The role of "Transcriptomics" in the search for new genes.

20

Principles of using CRISPR / Cas9-dependent counter-selection for precision editing of the genome of microorganisms. Prospects for using bacteria for ME.

Metabolic regulation of enzymes in nature - prevalence, role, target. Why does a large proportion of enzymes in a cell normally work at a fairly low level of their potential activity?

21

Synthetic biology for ME. Examples of synthetic genetic elements, structures, cascade regulators, genes, genomes. - Metabolic grafting, Retrosynthesis.

Modern methods for studying the structure and function of unknown genes and their protein products. Why is there still a large number of genes with unknown function in the genomes of even the most studied objects (for example, E. coli)?

22

What is orthogonal gene expression, "key players" of this system, and what is the difference between orthogonal and alternative expression systems.

Modern Methods of Metabolism Reconstruction from the Sequenced Genome: Theoretical Possibilities, the Need for Experimental Refinement and Verification.

Assessment "excellent (10)" is given to a student who has displayed comprehensive, systematic and deep knowledge of the educational program material, has independently performed all the tasks stipulated by the program, has deeply studied the basic and additional literature recommended by the program, has been actively working in the classroom, and understands the basic scientific concepts on studied discipline, who showed creativity and scientific approach in understanding and presenting educational program material, whose answer is characterized by using rich and adequate terms, and by the consistent and logical presentation of the material;

Assessment "excellent (9)" is given to a student who has displayed comprehensive, systematic knowledge of the educational program material, has independently performed all the tasks provided by the program, has deeply mastered the basic literature and is familiar with the additional literature recommended by the program, has been actively working in the classroom, has shown the systematic nature of knowledge on discipline sufficient for further study, as well as the ability to amplify it on one's own, whose answer is distinguished by the accuracy of the terms used, and the presentation of the material in it is consistent and logical;

Assessment "excellent (8)" is given to a student who has displayed complete knowledge of the educational program material, does not allow significant inaccuracies in his answer, has independently performed all the tasks stipulated by the program, studied the basic literature recommended by the program, worked actively in the classroom, showed systematic character of his knowledge of the discipline, which is sufficient for further study, as well as the ability to amplify it on his own;

Assessment "good (7)" is given to a student who has displayed a sufficiently complete knowledge of the educational program material, does not allow significant inaccuracies in the answer, has independently performed all the tasks provided by the program, studied the basic literature recommended by the program, worked actively in the classroom, showed systematic character of his knowledge of the discipline, which is sufficient for further study, as well as the ability to amplify it on his own;

Assessment "good (6)" is given to a student who has displayed a sufficiently complete knowledge of the educational program material, does not allow significant inaccuracies in his answer, has independently carried out the main tasks stipulated by the program, studied the basic literature recommended by the program, showed systematic character of his knowledge of the discipline, which is sufficient for further study;

Assessment "good (5)" is given to a student who has displayed knowledge of the basic educational program material in the amount necessary for further study and future work in the profession, who while not being sufficiently active in the classroom, has nevertheless independently carried out the main tasks stipulated by the program, mastered the basic literature recommended by the program, made some errors in their implementation and in his answer during the test, but has the necessary knowledge for correcting these errors by himself;

Assessment "satisfactory (4)" is given to a student who has discovered knowledge of the basic educational program material in the amount necessary for further study and future work in the profession, who while not being sufficiently active in the classroom, has nevertheless independently carried out the main tasks stipulated by the program, learned the main literature but allowed some errors in their implementation and in his answer during the test, but has the necessary knowledge for correcting these errors under the guidance of a teacher;

Assessment "satisfactory (3)" is given to a student who has displayed knowledge of the basic educational program material in the amount necessary for further study and future work in the profession, not showed activity in the classroom, independently fulfilled the main tasks envisaged by the program, but allowed errors in their implementation and in the answer during the test, but possessing necessary knowledge for elimination under the guidance of the teacher of the most essential errors;

Assessment "unsatisfactory (2)" is given to a student who showed gaps in knowledge or lack of knowledge on a significant part of the basic educational program material, who has not performed independently the main tasks demanded by the program, made fundamental errors in the fulfillment of the tasks stipulated by the program, who is not able to continue his studies or start professional activities without additional training in the discipline in question;

Assessment "unsatisfactory (1)" is given to a student when there is no answer (refusal to answer), or when the submitted answer does not correspond at all to the essence of the questions contained in the task.

## 5. Methodological materials defining the procedures for the assessment of knowledge, skills, abilities and/or experience

The course is graded at an exam. The questioning starts with a random task assigned to each student and time given for completion of the task. No aids are allowed. The student then proceeds to a chat with the examiner, at which he/she presents his/her solution to the assigned task. The examiner then asks the student several questions that evenly cover the course content. A final grade is assigned based on the quality of answers and demonstrated level of understanding.