**Al-FARABI KAZAKH NATIONAL UNIVERSITY**

**Approved**
**at the meeting of the**
**Academic Council Committee (SMC)**
**Al-Farabi KazNU**
**Vice-rector for academic affairs**
**_____A. K. Khikmetov**
**Protocol N6 from " 22 " 06 2020**

# PROGRAM
# ENTRANCE EXAM FOR APPLICANTS TO THE PHD-DOCTORAL PROGRAM IN THE EDUCATIONAL PROGRAM 8D05107-PHYTOBIOTECHNOLOGY

**ALMATY 2020**

The program is compiled in accordance with the State educational standard for the specialty 6D070100-Biotechnology.

The program was made up of: Doctor of Biological Sciences, professor Atabayeva S. D., Doctor of Biological Sciences, professor Kenzhebayeva S. D., Candidate of Biological Sciences, acting professor Asrandina S. Sh.

The program was reviewed at the meeting of the Department of biotechnology
Protocol no. _ _ _ _ _ _ from_____2020 .

Head of Department_____ Kistaubayeva A. S.

Approved at the meeting of the faculty's Method Bureau
Protocol no. _ _ _ _ _ from_____2020.
The Chairman of the Methodical Bureau_____ O. Jurikova

Approved at the meeting of the Academic Council
Protocol no. _ _ _ _ _ from _____ 2020.

Chairman of the Academic Council,
Dean of the faculty _____ B. K. Zayadan

Scientific Secretary _____ M. Bauenova

**CONTENT**

**1. Goals and objectives of the entrance exam in the specialty "8D05107-Phytobiotechnology»**

The goal of the entrance examination for admission to doctoral studies, specialty "Phytobiotechnology" is the determination of the level of theoretical and practical knowledge acquired, being trained in a magistracy, to evaluate compliance with the universal competence of the applicant, necessary for successful development of special competencies that are formed in the process of training for the doctoral education programme and obtaining the academic degree "Doctor of philosophy (PhD)» according to the educational program " 8D05107-Phytobiotechnology»

The task of the exam is to assess the ability and readiness of future doctoral students to search, select and synthesize information; to assess the readiness of applicants to use modern information resources in the learning process, the ability to use the knowledge obtained in solving scientific and industrial problems; skills in the practical use of various methods of biotechnology, to teach at universities, to successfully carry out research and management activities in scientific organizations and in production.

The exam form is written.

**2. Requirements for the level of training of persons entering the PhD program in the specialty " 8D051__ - Phytobiotechnology»**

The previous minimum level of education for people who want to master the educational programs of doctoral studies is the master's degree. Applicants to the doctoral program must have general professional competencies corresponding to the level of master's training, be able to formulate and study new problems from various fields of modern biotechnology, be able to organize their work on a scientific basis, and use their knowledge in laboratory and industrial conditions.

**3. Prerequisites of educational program**

"Modern methods in biotechnology", "Molecular and biochemical markers of plant resistance to diseases", "Current problems of gene expression", "Intracellular signaling", "Biotechnology of production of biologically active substances", "Biotechnology of agricultural plants", "Genetic bases of Phytopathology"

**4. List of exam topics**

1. Cell signaling systems. Types of regulation systems. Plant cell receptors. Intracellular and intercellular systems of regulation.

2. Membrane receptors. Types of membrane receptors. G-protein. Phospholipases associated with G-protein receptors, GPCR. Ion channels-receptors. Receptors that interact with enzymes ("anchor").

3. Perception and transduction of the signal. Signal transduction. Secondary messengers. Protein and non-protein messengers

4. Components of signal transduction. Signal transmission. Phosphoinositol pathway. Calmodulin role in the activation of protein kinases, Proteinkinase protein kinase and phosphatase. MAR-kinase cascade.

5. Metabolic and membrane regulation. Biochemical and biophysical mechanisms. Classic pH-stat. The main functions of cell membranes. Membrane system of regulation.

6. Gene regulation. The levels of signal transduction. Regulation at the transcription level. The level of translation. Protein level. Mechanisms of interaction of protein transcription factors of the cytoplasm with regulated DNA sites.

7. Functional and regulatory stress proteins. Regulatory and functional ABC-dependent stress proteins. Perception and transduction of the hormonal signal.

8. Trophic system of regulation. Bioelectrogenesis. The system of trophic regulation. Electrophysiological regulation system.

9. The system of regulation in stress conditions. The role of perception and transduction of a stress signal in the activation of the Stress protein genome. Warm shock proteins.

10. General concepts of phytopathology. Classification of plant diseases. Plant disease and pathological process. Classification of plant diseases. non-infectious and infectious diseases of plants. Types of pathogens. Response actions of plants to pathogens. Types of plant-pathogen interaction. The "gene-on-gene" theory of flora assumes that specificity is controlled by a pair of host and parasite genes and in most cases manifests itself in the interaction of the host's dominant resistance gene (R) with the dominant virulence gene (A) of the pathogen that overcomes the plant's resistance.

11. Mechanisms that determine plant resistance to pathogens. Anatomical, biochemical, physiological and molecular aspects that contribute to the development and maintenance of plant resistance, features of virulence factors.

12. Plant resistance to pathogens - a consequence of the interaction of host and parasite genes and the conditions for its occurrence. The role of a specific allele of the resistance gene (R-gene), the product of which recognizes a pathogen that has the corresponding allele of the avirulent gene (avr-gene). All other combinations of R-and avr-genes are not active against the development of the disease.

13. Resistance Genes in plants — R-genes. Sequences of R-gene nucleotides that determine resistance to different types of pathogens (viruses, bacteria, fungi), the similarity of which indicates the same nature of the occurrence of signaling events in the formation of a protective reaction.

14. The main classes of resistance genes in plants are R-genes. Direct relationship of R-gene diversity with their genomic organization. One of four states of different R-loci. The presence of a single R-gene with multiple alleles that determine resistance to different races of the pathogen.

15. Products of R gene expression. Features of proteins encoded by R genes containing a repeat rich in leucine, as well as protein kinase and nucleotide binding domains. Function of a protein domain containing a repeat rich in leucine, responsible for protein-to-protein binding and pathogen recognition. Connection of plant defense mechanisms with the formation of the elicitor-receptor complex.

16. Types of parasitism in microorganisms. The main groups of organisms: obligate saprophytes, facultative parasites, facultative saprophytes and obligate parasites, as a result of the evolution of parasitism. Mechanisms of influence on affected tissues depending on the type of parasitism. The mechanisms of pathogenicity.

17. The pathological process. Pathological process: the period before the penetration of the pathogen; the penetration of the pathogen into the plant; the spread of the pathogen in the tissues of the host plant; the manifestation of external signs of the disease.

18. Mechanisms of plant protection. Passive and active plant defense mechanisms. Factors of passive immunity: anatomical and morphological features; chemical composition of plants; osmotic pressure of cells; phytoncides, etc. Factors of active immunity: hypersensitivity, phytoalexins, phagocytosis, etc.

19. Parasitic specialization. Types of pathogen specialization: phylogenetic, histotropic, organotropic, and ontogenetic. Pathogens are highly specialized (monophages) and widely specialized (polyphages). The concept of physiological races. Ways of occurrence of physiological races.

20. Variability of plant pathogens. Variability of pathogens as the basis for the formation of new pathogenic forms. Mechanisms of variability in fungi, bacteria, and viruses.

21. Genetics of relationships between host plants and their parasites. Genetics of relationships between host plants and their parasites. Theory of conjugate evolution of parasite and host in their joint homeland. The theory of flora "gene for gene". Types of plant resistance to pathogens. Monogenic and polygenic stability. Convergent and multilinear varieties.

22. Main directions in breeding for disease resistance. Methods of screening for immunity: assessment of the extent and intensity of the lesion; the role of infectious backgrounds in the assessment of disease resistance.

23. Plant immunity to insect pests. Forms of food relations between phytophages and forage plants. Plants as a habitat for harmful organisms. The phytophage-plant system and its evolution. Factors of plant immunity: rejection or selection of plants by pests; antibiosis; plant resistance to damage. The system of immunogenetic barriers: constitutional, induced.

24. Genetic bases of plant immunity to pests. Polymorphism. Ecological and genetic structure of phytophagous populations. Biological races (biotypes). Principles and methods for detecting plant resistance to phytophages.

25. Clonal micro-propagation and plant health improvement. Methods of clonal micro-propagation of plants, stages of micro-clonal reproduction, factors affecting the process of micro-clonal reproduction, improvement of planting material from viruses.

26. Bridging in vitro and progamous and postgamous incompatibility. Progamous and postgamous incompatibility in distant hybridization. In vitro fertilization. Culture of isolated zones. Culture of endosperm

27. Haploid technology. Anther culture. The use of haploproducers and distant hybridization in obtaining haploid tissues. Obtaining haploid plants in the culture of female gametophytes. Features of haploid technologies.

28. Cellular engineering. Culture of protoplasts. Isolation and production of viable protoplasts. Cultivation of protoplasts. Plant regeneration in protoplast culture.

29. Somatic hybridization. Principles of somatic hybridization. Genetic bases of somatic hybridization. Somatic hybridization of remote plant species. Methods of selection of somatic hybrids. Methods of analysis of hybrid plants. Practical application of somatic hybridization.

30. Cell selection. Methods of cell selection. Selection of resistant cells. Stability of the stability attribute. Induced mutagenesis. Features of mutagenesis and selection of mutants in vitro. Influence of mutagens on the survival of cultured in vitro cells. Methods of selection of cellular variants.

31. Somaclonal variants. Somaclonal variability. Natural genetic diversity of plant cells. Genome variability during in vitro culture. Variability of cytoplasmon from somaclonal variants. The value of the genotype and the original explant. Influence of conditions of cultivation and regeneration of plants. Genetic analysis of somaclons. Practical use and prospects of application of somaclonal variability.

32. Genetic engineering of plants. Transformation of plants by Ti-plasmid from *Agrobacterium tumefaciens*. Vector systems based on Ti-plasmids. Methods of gene transfer to plant cells. Application of reporter genes in the transformation of plant cells. Selection of various promoters and their use. Introduction of foreign genes into chloroplast DNA. Obtaining transgenic plants that do not contain marker genes.

33. Application of plant genetic engineering. Breeding of plants resistant to insect pests, viruses, herbicides, fungi and bacteria.

34. Obtaining plants that are resistant to various stress factors and aging. Oxidative stress, salt stress. Fruit ripening. The use of phytopathogen toxins in the selection of plant forms that are resistant to diseases. Isolation of salt-resistant plant forms by direct and indirect selection in tissue culture. Selection of cold-resistant forms.

35. Classification of products of biotechnological productions. Natural macromolecules – proteins, enzymes, hormones, vitamins, polysaccharides, polyesters, antibiotics, biogenic stimulants, pesticides isolated from the cells of microorganisms, tissues and organs of plants and animals.

36. Basic principles of protein production and methods of their purification. Use of microorganisms (yeast, bacteria, algae, fungi) for protein production. Methods of protein purification. Preparation of the extract cell Destruction and extraction. Optimization and clarification of the extract. Methods used for cleaning proteins and enzymes associated with particles.

37. Methods for isolating biologically active substances from plant material. Features of extraction from plant raw materials with a cellular structure. Extraction stages and their quantitative characteristics. The main factors affecting the completeness and speed of extraction. Requirements for the extractants.

38. The main types of extraction (maceration, percolation, repercolation, accelerated fractional maceration by countercurrent method, circulation extraction, continuous countercurrent extraction with mixing of raw materials and extractant, extraction with liquefied gases). Intensification of extraction processes (extraction using a rotary pulsating device, using ultrasound, using electric discharges, using electroplasmolis and electrodialysis).

39. Industrial production of biologically active substances from plant cell culture. Preparation of the medium for cultivation of the producer and seed material. Biosynthesis of biologically active substances. Separation, purification of biologically active substances and obtaining of finished products.

40. Biotechnology for the production of enzymes. Scope and sources of enzymes. Selection of the strain and cultivation conditions. Technology of cultivation of microorganisms - producers of enzymes and isolation of enzymes. Isolation and stabilization of enzymes. Application of microbial enzymes.

41. The production of amino acids. Biotechnology of amino acid synthesis and purification. Obtaining of amino acids using immobilized cells and enzymes. The production of optical isomers of amino acids by use of microorganisms acylases.

42. Production of vitamins. General characteristics of vitamins. Getting water-soluble) vitamins (vitamin B1, B2, B6, BC, PP, B3, B12, vitamin C). Obtaining fat-soluble vitamins(ergosterol, vitamin D2). Obtaining of carotenoids.

43. Production of organic acids. Preparation of organic acids (citric, lactic, acetic, propionic, itaconic, gluconic, fumaric acid) for use in the food and pharmaceutical industry, in engineering and as chemical raw materials.

44. Principles of technical equipment of bio-production facilities. And instrumentation used for microbiological productions. Management of technological processes of biosynthesis of biologically active substances. Waste from biotechnological industries and their neutralization and disposal.

45. Practical use of modern methods in agriculture, industrial biotechnology, and the development of new products.

46. Stages of methods used for subcellular fractionation. Methods of purification and identification of subcellular fractions. Differential centrifugation and its use. Separation of subcellular components. Identification of cellular components and criteria for their purification.

47. Modern methods of studying membrane structures. Methods for determining the lipid composition. Membranes and detergents. The principles of solubilization of the membranes. The use of detergents in the study of cell membranes. Modern types of biophysical methods for studying membrane structures.

48. Solubilization and reconstruction of membrane structures. Criteria for choosing detergents, their characteristics. Methods for isolation and modification of membrane proteins and peptides. Separation and analysis of lipid components of membranes. Identification of lipid components of membranes. Methods of isolation and identification of fatty acids. Types of chromatography used for the quantitative determination of fatty acids. Their advantages and disadvantages.

49. Analysis and characterization of proteins. Principles of protein isolation from biological objects. Factors affecting protein stability. Methods for isolating proteins from

membrane structures. Calorimetric methods of protein research. Spectral methods of protein research. The main criteria for the purity of protein preparations. Quantitative methods for determining proteins.

50. Physical and biophysical methods used to study membrane systems. Spectral methods for studying stationary properties of biological systems. Electron and paramagnetic resonance method, nuclear magnetic resonance. Methods for studying the ion permeability of biological membranes.

51. Proteomic methods for studying proteins. Methods for isolation and purification of proteins. Centrifugation, salt fractionation, gel filtration, dialysis. The types of membrane filtration for isolation of proteins. Ultrafiltration methods, reverse phase chromatography, distribution chromatography, gel chromatography. Gel electrophoresis. Isoelectric focusing analysis. Mass spectrometric method for protein analysis

52. Methods of isolation and analysis of nucleic acids. The main criteria for their purity. Quantitative determination of nucleic acids. Selection of methods for the analysis of nucleic acids. Methods for isolating RNA from biological objects. Basic methodological techniques. Quantitative analysis of nucleic acids.

53. Modern methods of nucleic acid sequencing. Stages and types of nucleic acid sequencing methods. Principles of radioautography.

54. The principle of polymerase chain reactions (PCR). Methods of nucleic acid hybridization. Conditions for hybridization, selection of probes. Blot hybridization method. Principle of the method, stages, components of the reaction. Necessary equipment for PCR. Varieties of polymerase chain reactions (PCR). Using polymerase chain reactions to analyze the primary structure of nucleic acids. Use of PCR.

55. Methods of genetic engineering. The concept of recombinant structure. A mechanism for the creation of recombinant DNA. Expression of transferred genes. Methods for cloning recombinant DNA molecules. Practical and commercial applications of genetic engineering. Obtaining transgenic plants and animals.

56. Methods for creating recombinant DNA molecules. Enzymes of the metabolism of nucleic acids used in genetic engineering. Characteristics of restriction enzymes, their classification. Isoschizomers. Restriction maps and restriction fragments. Methods for constructing a recombinant DNA molecule, obtaining a cDNA gene, restriction, ligation, and methods for transferring genes to cells of various organisms.

57. Methods for cloning recombinant DNA molecules. General characteristics of bacterial plasmids as autonomously replicating minichromosomes. Episomes, nontransmissible plasmids. The number of copies of the plasmid in the cell. Other host vector systems: bacteriophage λ, cosmids, bacteriophage M13. Probes for the detection of cloned genes. Identification of specific cDNA clones using nucleic acid hybridization.

58. Methods for selecting cloned genes. Selection of clones of bacteria that have received recombinant plasmids, using genes that determine resistance to antibiotics (inactivation as a result of insertion). Southern and northern blotting. Screening of gene libraries using oligonucleotide probes. Enzymatic, immunological and enzyme immunoassay (ELISA) methods for identifying protein products of genes and nucleic acids proper (digoxygenin, triple helix of nucleic acids). Using the polymerase chain reaction (PCR) method for identification, amplification and isolation of specific sections of DNA.

59. Plant viruses as vectors for genetic engineering. Classification of plant viruses by type of their genetic material. Groups of geminiviruses and caulimoviruses as the most suitable for the role of genetic vectors. Characteristics of cauliflower mosaic virus (CaMV) as a typical representative of the caulimovirus group. Regions of the CaMV genome that are most suitable for introducing foreign DNA. Techniques for transforming plants with vectors based on the CaMV virus. The main advantages and disadvantages of CaMV-based vectors.

60. Methods of breeding the cloned recombinant DNA. Selection of clones of bacteria that have received recombinant plasmids, using genes that determine antibiotic resistance

(inactivation as a result of insertion). Reporter genes used as markers for the selection of transformed bacterial clones.

61. Methods of transformation of plant protoplasts, cells and tissues. Plasmids that induce tumors induced by certain soil bacteria. Genetic engineering of plants. Crown galls are plant tumors. Tumour-inducing plasmids (Ti-plasmids). Ti-plasmid mutants. Integration of T-DNA with the plant chromosome. Ti-plasmid DNA as a vector. Transformation of plant cells and protoplasts. Mobilization of T-DNA using the VIR segment of the Ti-plasmid. Attenuated t-DNA vectors make it possible to regenerate an entire plant from a single cell. Embedding T-DNA can be used to isolate plant genes. Practical application of plant genetic engineering using Ti-plasmids.

62. Advantages of the eukaryotic cloning system for genetic research and for studying the regulation of eukaryotic gene expression on the example of yeast cells. Yeast spheroplasts. Expression of yeast genes in *E. coli* bacteria. Shuttle vectors. Yeast plasmids. Increase the efficiency of transformation by using additional replication start points (Autonomous replication elements, ear). Stabilization of yeast plasmids by introduction of centromeric (CEN) yeast DNA. The hairpins at the ends of yeast chromosomes are telomeres. Directed integration of cloned DNA into chromosomes of yeast. Organization and regulation of gene expression in yeast.

63. Stages of gene expression. The functional product of gene expression is RNA or protein. Activation of gene expression by short double-stranded RNAS. Regulation of the stages of gene expression: transcription, translation, RNA splicing, and the stage of posttranslational modifications of proteins.

64. Regulation of gene expression is the basis for cell differentiation, morphogenesis, and adaptation. The influence of factors on the quantitative characteristics of the expression of one gene and the function of other genes in the whole body.

65. Regulation of MicroRNA gene expression. Characteristics of MicroRNAs and their mechanism of action. The influence of specific MiRNAs on gene expression.

66. Determination of gene expression. Basic methods for determining gene expression. Basic principles of RNA sequencing.

67. Sequencing of RNA containing poly-A (mRNA) and expression DNA microchips. Advantages of RNA sequencing by next-generation sequencing to identify different mRNA variants resulting from alternative splicing.

68. Application of real-time reverse transcription PCR (quantitative PCR, Real-time PCR, qPCR, qRT-PCR) - to determine gene expression.

69. Application of real-time PCR for detection of genetically modified organisms (GMOs) based on specific primers of amplification of the promoter, terminator, or even intermediate sequences used in the process of creating the vector.

70. Repressor-a DNA-binding or RNA-binding protein that inhibits the expression of one or more genes. Mechanism of action of the repressor on gene expression.

**List of recommended literature**

**The main references:**

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**Criteria for evaluating knowledge in the specialty 8D05107-Phytobiotechnology, PhD doctorate**

| Rating by letter system | Digital equivalent of points | % content | Rating by traditional system | Competency scale |
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|---|---|---|---|---|
| A | 4,0 | 95-100 | excellent | This rating is given if the applicant:<br>- has deep theoretical and practical knowledge in the areas of biotechnology;<br>- has knowledge of modern methods used in the field of biotechnology; understanding the essence and relationship of the considered biotechnological processes;<br>- has the skills to process and analyze data, use them in research and calculations;<br>- has the basics of management, has the skills to analyze the primary experimental data of the study of the structure and physical and chemical properties of biotechnological objects using the main methods;<br>- provides correct, logically consistent, complete and specific answers to all questions on the exam ticket. |
| A- | 3,67 | 90-94 | | Данная оценка ставиться в том случае, если претендент:<br>- владеет хорошими навыками использования теоретических и практических знаний по направлению биотехнологии;<br>- владеет знаниями по современным методам используемых в области биотехнологии;<br>- Понимает суть и взаимосвязи рассматриваемых биотехнологических процессов;<br>- дает последовательные и конкретные ответы на поставленные вопросы при свободном устранений замечаний по отдельным и частным аспектам ответов. |
| B+ | 3,33 | 85-89 | good | This rating is given if the applicant:<br>- has good skills of using theoretical and practical knowledge in the field of biotechnology;<br>- has knowledge of modern methods used in the field of biotechnology; |

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|---|---|---|---|---|
| | | | | - understands the essence and relationship of the considered biotechnological processes;<br>- provides consistent and specific answers to the questions raised, with free elimination of comments on individual and particular aspects of the answers. |
| B | 3,0 | 80-84 | | This rating is given if the applicant:<br>- has simple skills of using theoretical and practical knowledge in the areas of biotechnology;<br>- has knowledge of modern methods of biotechnology;<br>- gives correct answers to questions. |
| B- | 2,67 | 75-79 | | This rating is given if the applicant:<br>- has incomplete knowledge of theoretical and practical knowledge in the areas of biotechnology and understanding of the main issues of the program;<br>- non-specific, without gross errors answers to the questions raised when eliminating inaccuracies and errors in leading questions of members of the commission. |
| C+ | 2,33 | 70-74 | satisfactory | This rating is given if the applicant:<br>- has incomplete knowledge of theoretical and practical knowledge in the areas of biotechnology;<br>- lack of understanding of the main issues of the program;<br>- non-specific, without gross errors answers to questions when eliminating inaccuracies and errors in the leading questions of examiners. |
| C | 2,0 | 65-69 | | This rating is given if the applicant:<br>- incorrect answer to at least one of the main questions:<br>- gross errors in the response, lack of understanding of the essence of the problems presented;<br>- uncertain and inaccurate answers to additional questions |