

## OPINION

by **Prof. Ivan Iliev Atanasov, AgroBioInstitute, Sofia, AA**

of a dissertation for awarding the educational and scientific degree '**doctor**'

in the field of higher education: **4. Natural sciences, mathematics and informatics**

professional direction: **4.3 Biological sciences**

PhD program: **Genetics - Bioinformatics**

Author: **Georgi Yordanov Miloshev**, full-time doctoral student at the Department of Genetics, Faculty of Biology, Sofia University "St. Cl. Ohridski".

Title: **Design and application of functional nucleic acids for synthetic control of gene expression.**

Research supervisor: **Prof. Dr. Robert Penchovski**, Faculty of Biology, Sofia University "St. Cl. Ohridski".

### **1. General description of the presented materials**

By order RD 38-469 of 21.07.2023 of the Rector of Sofia University "St. Cl. Ohridski" I have been appointed as a member of the scientific jury in the procedure for the defense of a dissertation work on the topic "Design and application of functional nucleic acids for synthetic control of gene expression" for the acquisition of the educational and scientific degree "doctor" in the field of higher education: 4. Natural sciences, mathematics and informatics, professional direction: 4.3 Biological sciences, doctoral program: Genetics - bioinformatics. The author of the dissertation is Georgi Yordanov Miloshev, is a full-time doctoral student at the Department of Genetics, Faculty of Biology, SU "St. Cl. Ohridski" with supervisor Prof. Dr. Robert Penchovski, Faculty of Biology, SU "St. Cl. Ohridski". The set of materials for the procedure presented by Georgi Yordanov Miloshev is in accordance with the Regulations for the Development of the Academic Staff of SU "St. Cl. Ohridski".

### **2. Brief biographical data for the doctoral student**

Georgi Yordanov Miloshev was born in 1994. In 2016, he received a master's degree in "genetics and genomics" from the Faculty of Biology, SU "St. Cl. Ohridski", and since 2019 he has been enrolled as a full-time doctoral student at the Department of Genetics, Faculty of Biology, SU "St. Cl. Ohridski" and graduated with the right to defense in 2022. Since 2019, he has been a teacher at 125 SU "Boyan Penev", and since 2022 he has been teaching at the "Innovation" Educational Center, Sofia. He speaks very good English and mainly French and Russian. During the doctoral studies, he

participated in the collectives and the implementation of a total of three projects financed by the "Scientific Research" Fund of the Ministry of Education and Culture, including research in the field of dissertation work. He is the co-author of four publications in the field of doctoral studies. Georgi Miloshev participated in a large number of training and educational courses mainly in the field of biology and pedagogy. He is the recipient of the "Doctoral Student of the Year" award (2022) of SU "St. Kliment Ohridski", as well as awards for pedagogical activity of 125 SU "Boyan Penev" and the Regional Department of Education - Sofia.

### **3. Relevance of the topic and appropriateness of the set goals and tasks**

The construction and application of synthetic antisense oligonucleotides for control of gene expression in microorganisms is a dynamically developing direction of molecular and synthetic biology with expected applications in various fields of medicine and bioindustry. Research within the presented dissertation work includes the design and experimental testing of ACO to control/inhibit the expression of the LacZ reporter gene in *Escherichia coli*. The study is exemplary and the experience and results can be successfully applied in subsequent studies to use ASO to control and inhibit the expression of other genes and in other types of microorganisms. This makes the research included in the dissertation work and obtained results up-to-date, original, with a positive effect and providing a basis for further applications in various fields.

### **4. Knowing the problem**

The dissertation includes a rich, comprehensive and well-illustrated literature review on the main topics and problems of the dissertation, including: identification of indicator RNAs, design and applications of allosteric ribozymes, use of ASOs to control gene expression, and consideration of perspectives in RNA development synthetic biology. All this, together with the discussions of the obtained experimental results and perspectives for the applications of ASO, demonstrates the doctoral student's in-depth awareness and knowledge of the complex issues related to the topic of the dissertation work.

### **5. Research methodology**

Two main groups of methods were used in the dissertation work: bioinformatics methods including the use of specialized software (Clustal X, BLAST, RNAfold web server, Motif searcher, etc.) and databases (Rfam 14.8, KEGG, etc.) for analysis and work with nucleotide sequences and microbiological and molecular genetic methods including established laboratory procedures for recombinant DNA, *E.coli* transformation, etc.

## **6. Characterization and evaluation of the dissertation work**

The dissertation work is presented in the generally accepted style on 131 pages and includes a total of 61 figures and 4 tables, and some of the figures are presented in color, which further contributes to their informativeness, good illustration and clear presentation of information. The literature review is rich and includes a significant amount of literature data and well-systematized discussions on the main topic of the dissertation work. The purpose and tasks of the dissertation work are clearly formulated. Methodology. In the dissertation research, the doctoral student uses a wide range of bioinformatics methods, use of specialized software and databases, as well as the necessary set of microbiological methods. The materials and methods used are presented in the dissertation. Results. The obtained experimental results are presented in their logical sequence in a total of 6 parts: three parts ACO design and strategy to control LacZ gene expression and three parts obtaining and testing a construct to control gene expression. The presentation of the results in each of these parts includes a discussion of the specific results. It is unclear to me the need to include separate figures for the effect of each ACO concentration tested on LacZ gene expression (these are figures numbered №48-№56) after information about these results is summarized in Figure 57. After the results part the dissertation also includes a separate part 'Discussion' (3 pages) including a summary of the main results. Conclusions and contributions. In my opinion, some of the presented conclusions can be better formulated, arranged and edited in order to better present the thesis work in a logical and coherent manner. Similarly, I believe that the contributions formulated in this way can be further specified. The cited literature includes a total of 184 literary sources in English and shows the high awareness and knowledge of the doctoral student regarding the level of research on the topics of the dissertation work.

## **7. Contributions and significance of the development for science and practice**

The results presented in the dissertation work are original, of high significance for the field of the dissertation work and with real prospects for further applications. Original scientific contributions include selection of the ACO sequence and experimental confirmation of the effectiveness of its application to control the expression of the LacZ reporter gene in *Escherichia coli*.

## **8. Assessment of dissertation publications**

Results of the dissertation work were published in two scientific publications in refereed journals (*Proceedings of the Bulgarian Academy of Sciences* 75 (1), 2022, pp. 56–61., 2022 and *Acta Microbiologica Bulgarica* Volume 39 (1), 2023, pp. 3–11). Part of the results are also presented in a

book chapter (*New Frontiers and Applications of Synthetic Biology, 2022, eBook ISBN: 9780323859868, p.103-121*).

### **9. Personal participation of the doctoral student**

The PhD student is first author on the main scientific publication on the dissertation and second author on the book chapter.

### **10. Abstract**

The abstract is made according to the requirements and fully presents the content of the dissertation work, including: goals and objectives; materials and methods; results and discussion; conclusions; contributions and publications related to the dissertation.

## **CONCLUSION**

The dissertation contains significant scientific results that represent an original contribution, have real prospects for further applications and meet all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria / ZRASRB/, the Regulations for the Implementation of ZRASRB and the relevant Regulations of the Sofia University "St. Kl. Ohridski". The presented materials and dissertation results correspond to the specific requirements of the Faculty of Biology, SU "Kl. Ohridski".

Taking into account the above, **I give my positive assessment of the research conducted by Georgi Yordanov Miloshev, presented by the above-reviewed dissertation work, abstract, achieved results and contributions, and I propose to the honorable scientific jury to award the educational and scientific degree "doctor" to Georgi Yordanov Miloshev in the field of higher education: 4. Natural sciences, mathematics and informatics, professional direction 4.3 Biological sciences, doctoral program Genetics - bioinformatics.**

28.09. 2021 г.

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Prof DSc Ivan Atanassov