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Skolkovo Institute of Science and Technology

Jury Member Report – Doctor of Philosophy thesis.

Name of Candidate: Daria Artamonova

PhD Program: Life Sciences

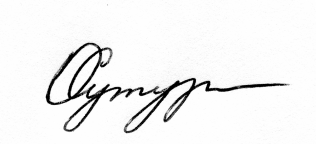
Title of Thesis: Comparative Analysis of the Action of Eubacterial Class 1 CRISPR-Cas Systems.

Supervisor: Professor Konstantin Severinov

Chair of PhD defense Jury: Professor Yuri Kotelevtsev ***Email:*** ***y.kotelevtsev@skoltech.ru***

Date of Thesis Defense: October 24, 2017

Name of Reviewer: Olga SOUTOURINA

I confirm the absence of any conflict of interest (Alternatively, Reviewer can formulate a possible conflict)	**Signature:**  **Date: 22-09-2017**

The purpose of this report is to obtain an independent review from the members of PhD defense Jury before the thesis defense. The members of PhD defense Jury are asked to forward a completed copy of this report to the Chair of the Jury at least 30 days prior the thesis defense. The Reviewers are asked to bring a copy of the completed report to the thesis defense and to discuss the contents of each report with each other before the thesis defense.

If the reviewers have any queries about the thesis which they wish to raise in advance, please contact the Chair of the Jury.

Reviewer's Report

Reviewers report should contain the following items:

- Brief evaluation of the thesis quality and overall structure of the dissertation.
- The relevancy of the topic of dissertation work to its actual content
- The relevancy of the methods used in the dissertation
- The scientific significance of the results obtained and their compliance with the international level and current state of the art
- The relevance of the obtained results to applications (if applicable)
- The quality of publications
- The summary of issues to be addressed before/during the thesis defense

The work of Daria N. Artamonova, performed under the scientific direction of Pr. Konstantin Severinov and Dr. Ekaterina Semenova focus on the comparative analysis of the action of bacterial class 1 CRISPR-Cas systems for defense against foreign nucleic acids and new spacer acquisition. The dissertation contains a large review of the literature on the all steps of CRISPR-Cas system activity in bacteria, in particular crRNA biogenesis, adaptation and interference. Interesting updated aspects of CRISPR-Cas systems diversity and evolution, as well as discovery of the anti-CRISPR genes are also discussed. This introduction is extremely complete, well structured and very well illustrated.

In a first part, Daria Artamonova gives an historical view on the discovery of CRISPR-Cas systems and general mechanisms for protection against foreign invaders and avoiding of autoimmunity. Daria Artamonova describes the current state of knowledge about the different stages of CRISPR-Cas system functioning. In particular, to introduce her work, Daria Artamonova reviewed the general characteristics of the class 1 CRISPR-Cas systems by focusing on the systems on which she works, namely Type I and Type III systems. An interesting analysis underlines the connections between interference and primed adaptation mechanisms.

The second chapter is devoted to the studies of adaptation in I-F subtype CRISPR-Cas system and organized as a scientific article. This part of the results corresponds to an article published in the Journal "***Nucleic Acids Research***" in 2015 that Daria Artamonova signs as the first author. The description of this part of the experimental thesis work begins with a brief overview of the characteristics of the I-F subtype CRISPR-Cas system and the materials and methods used. Daria Artamonova clearly underlines in this part the important questions that remain to be addressed and formulates the relevant objectives of the thesis work. Before presenting the results, the experimental strategy is described including the establishment of the model of I-F system from *Pseudomonas aeruginosa* in heterologous host *Escherichia coli*. Using this experimental model the genetic requirements for spacer acquisition by the I-F *P. aeruginosa* system were identified. The results suggest that all components of CRISPR-Cas machinery are needed both for primed and naïve adaptation in type I-F system. Anti-CRISPR proteins previously shown to inhibit the interference function of CRISPR system prevented also its adaptation activity. High throughput sequencing approaches were used to identify the origin and the distribution of the acquired spacers. In addition to type I-F system from *P. aeruginosa*, the spacer acquisition by the I-F system from *E. coli* ED1a strain was also investigated. At the end of this chapter the major findings of this work is discussed emphasising the differences observed for adaptation requirements in this system as compared to type I-E CRISPR-Cas from *E. coli*. Daria Artamonova has directly contributed to another publication on the primed spacer acquisition by *E. coli* type I-E system.

The third chapter describes the work on the type III-A and III-B CRISPR-Cas systems from *Thermus thermophilus*. After a brief introduction on the particularities of type III CRISPR-Cas systems and the materials and methods section, this part of the dissertation describes the attempts to detect the naive and primed adaptation in this system using various available assays with plasmids and synthetic nucleotides transformation and bacteriophage infection. No adaptation was observed in all tested conditions. To differentiate the action of type III-A and III-B systems the mutant strains were created lacking either one or both systems. The results demonstrate that the type III-A and III-B systems are both active for interference process and are resistant to a large number of spacer-protospacer mismatches, type III-B system being more sensitive to mismatches than type III-A. The results obtained during this work are then discussed and compared to the recent findings for other CRISPR-Cas systems of type III, II and I.

Finally, the major findings of the work are summarized in the comparative table describing the features of naive and primed adaptation as well as the position of the “seed” region for studied I-

E, I-F, III-A and III-B CRISPR-Cas systems.

In conclusion, the results of Daria N. Artamonova represent an important scientific contribution to better understand the mechanisms of bacterial adaptive immunity systems. Daria Artamonova (Vorontsova) signs an article published in international peer-reviewed journal "***Nucleic Acids Research***" in 2015 as the first author describing the first part of her work on the foreign DNA acquisition mechanisms by the type I-F CRIPSR-Cas system. Many results presented in the dissertation are promising and should bring to additional publications. Daria Artamonova also participated in an article on the primed spacer acquisition by type I-E CRISPR system published in a high-ranked journal "***Proceedings of the National Academy of Sciences***" in 2016 as a 5th author. The candidate presented her results during International conferences in Russia and Israel. The thesis work represents a high quality set that opens many perspectives for the future developments. The thesis manuscript is pleasant to read and clearly outlines the intellectual path and approaches used during the work. It is worth noting the impressive volume of work done by Daria Artamonova with a broad spectrum of *in vitro*, *in vivo* and *in silico* approaches. On the manuscript reading, it is clear that she has shown initiative and reflection to carry out her thesis work. I therefore consider that Daria Artamonova fully deserves to present her results in order to obtain a Ph.D. of Skolkovo Institute of Science and Technology.

Provisional Recommendation

☒ *I recommend that the candidate should defend the thesis by means of a formal thesis defense*

☐ *I recommend that the candidate should defend the thesis by means of a formal thesis defense only after appropriate changes would be introduced in candidate's thesis according to the recommendations of the present report*

☐ *The thesis is not acceptable and I recommend that the candidate be exempt from the formal thesis defense*