

Jury Member Report – Doctor of Philosophy thesis.

Name of Candidate: Sofia Medvedeva

PhD Program: Life Sciences

Title of Thesis: Natural diversity of CRISPR spacers

Supervisor: Prof. Konstantin Severinov

Co-Supervisor: Dr. Mart Krupovic

Chair of PhD defense Jury: Prof. Mikhail Gelfand

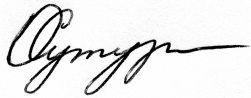
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Date of Thesis Defense: 3 June 2019

Name of the Reviewer: Pr. Olga Soutourina

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| I confirm the absence of any conflict of interest (Alternatively, Reviewer can formulate a possible conflict) | Signature:  Date: 03-05-2019 |
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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 submit signed copy of the report 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 relevance of the topic of dissertation work to its actual content
- The relevance 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 Sofia Medvedeva, performed under the scientific direction of Pr. Konstantin Severinov and Dr. Mart Krupovic focused on the analysis of natural diversity of CRISPR spacers in various microbial populations. The manuscript of 188 pages is organized in 5 parts including the introduction with the review of the literature, aims of study, results in 6 chapters and conclusions and future perspectives with appendix. The publication list of Sofia Medvedeva is provided at page 5 and the personal contribution of the candidate is specified before each results chapter. The dissertation contains a brief review of the literature on the defense systems in prokaryotes focusing on CRISPR-Cas system components and activities including adaptation and interference modules. In relation to the overall aim of the study the distribution of CRISPR-Cas systems is briefly presented as well as particular features of this defense system in Sulfolobales hyperthermophilic archaea. Some additional aspects of CRISPR-Cas system are shortly mentioned as its use for strain subtyping, metagenomics analysis, recent discovery of anti-CRISPR proteins and noncanonical functions of CRISPR-Cas. The candidate has chosen extremely condensed and synthetic presentation of the scientific literature. On my opinion, the literature review appears too short and some additional information could be provided together with related illustrations to facilitate the reading of the manuscript before the description of the results of these studies.

The objectives of this work are clearly stated before the results section. This section is organized in six chapters and describes the main findings on various subjects related to CRISPR spacer diversity and dynamics in different natural communities. There is no specific section devoted to the materials and methods used for these studies and their description is included to each specific paper. Thus each chapter corresponds to a published work or an article submitted for publication. I have some concerns on the presentation of this results section. On my opinion, some additional sentences for transitions between different papers should be helpful to link these different pieces of work together.

Overall, the presented studies show that exploration of natural diversity of CRISPR spacers from environmental samples provides valuable data on host-virus interactions that could not be uncovered only from available genomic sequences in databases. Surprisingly, the comparison of CRISPR spacer content between contemporary and ancient *Escherichia coli* arrays revealed a low activity of type I-E CRISPR-Cas adaptation module over 40, 000 year-period. The analysis of natural community of *Thermus*, *Sulfolobus* and *Flavobacteria* from different geographic zones and environments revealed CRISPR adaptation to local virus communities. Interesting example of two *Sulfolobus* viruses carrying mini-CRISPR arrays targeting competing virus is discussed with perspectives on the virus speciation.

Finally, the major findings of the work are summarized and discussed in the last conclusions and perspectives section highlighting the similarities and differences between various studied systems. The future directions could be probably discussed in more detail in this part.

In conclusion, the results of Sofia Medvedeva represent an important scientific contribution to better understand the CRISPR-related mechanisms of bacterial and archaeal interactions with viruses. Sofia Medvedeva signs an article published in international peer-reviewed journal "**Philos Trans R Soc Lond B Biol Sci**" in 2018 as the first co-author describing the diversity of CRISPR spacers of *Thermus* strains from different environmental samples. Sofia contributed as a first author for an article submitted for publication on the virus-borne mini-CRISPR arrays promoting interviral conflicts and viral speciation. She also participated in an article on the metagenomic analysis of bacterial communities from Antarctic snow published in a journal "**Frontiers in Microbiology**" in 2016 as a second author. Sofia also contributed as a third author to the paper on the dynamics of *Escherichia coli* type I-E CRISPR spacers from ancient mammoth sample published in "**Molecular Ecology**" in 2016 and to the paper on the efficiency of prespacer selection during primed adaptation published in "**mBio**" in 2018. She also participated as a 4th author in the work on the integrated mobile genetic elements in Thaumarchaeota published in "**Environmental Microbiology**" in 2019. The candidate presented her results during International conferences in Russia and abroad. 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 Sofia with a broad spectrum of *in silico* approaches contributing to several collaborative projects. On the manuscript reading, it is clear that she has shown initiative and reflection to carry out her thesis work. For all these reasons I consider that Sofia Medvedeva 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