

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

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Date of Thesis Defense: 3 June 2019

Name of the Reviewer: Tamara Basta-Le Berre

<p>I confirm the absence of any conflict of interest</p> <p>(Alternatively, Reviewer can formulate a possible conflict)</p>	<p>Signature:</p>  <p>Date: 04-05-2019</p>
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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

- Brief evaluation of the thesis quality and overall structure of the dissertation.

In course of evolution cells have acquired different defense mechanisms against the mobile genetic elements such as viruses and plasmids. One of these is the CRISPR-Cas system, a sort of adaptive immunity which protects the prokaryotic cells by keeping track of past encounters with viruses and plasmids. The discovery of the CRISPR-Cas adaptive immunity and its use as genome editing tool is one of the most important discoveries in biology in the last decade. The CRISPR array is composed of short repeat sequences and spacer sequences sandwiched between repeats. Repeat sequences are specific for a type of CRISPR-Cas system and of species which carries them while spacers correspond to viral or plasmid sequences. In her thesis work, Sofia Medvedeva has studied the diversity of CRISPR spacers and the dynamics of their acquisition in prokaryotic populations from different natural or laboratory environments. The theses manuscript contains a general introduction chapter, six chapters corresponding to original articles (five published papers and one submitted) and a general conclusion and perspectives chapter. Overall, the manuscript is of very good quality. The quantity, variety and quality of presented data is remarkable.

- The relevance of the topic of dissertation work to its actual content

The topics of the presented dissertation work are: the dynamics of CRISPR-spacer acquisition over forty thousand years in *E.coli* species; the diversity of CRISPR-spacers among bacteria living in antarctic surface snow; diversity of CRISPR-spacers in *Thermus* bacteria, in Sulfolobales archaea and in integrated mobile genetic elements of Thaumarchaea; and mechanism of protospacer selection during primed adaptation. Together, the content of the thesis work is thematically coherent and totally pertinent with respect to the announced topic of the thesis.

- The relevance of the methods used in the dissertation

The theses work was mainly based on bioinformatics and statistical analysis to extract, classify and analyse CRISPR spacer sequences from next generation sequencing data or databases. These are appropriate methods to study the diversity of CRISPR spacer sequences.

- The scientific significance of the results obtained and their compliance with the international level and current state of the art

The CRISPR spacer sequences are a catalogue of past viral infections and as such a valuable source of information on different aspects of virus/plasmid – host interactions. However, the natural CRISPR spacer sequences are still drastically undersampled. Thus, the novel CRISPR spacer sequence data provided by this theses work are much needed and significant contribution to the field. The analysis of these CRISPR spacer sequences allowed to reach several non-trivial conclusions. The confirmation was provided that the diversity of CRISPR spacer sequences greatly surpasses that of genomic sequences from cultivated species. The analysis of spacers from mammoth samples confirmed that the *E. coli* type I-E CRISPR-Cas system has been probably inactive for at least forty thousand years. The *Thermus*, *Sulfolobus* and *Flavobacterium* communities were shown to adapt to local viruses using different CRISPR-Cas systems. Curiously, while *Sulfolobus* and *Flavobacterium* communities were shown to display a biogeographical pattern, as established from their CRISPR spacer catalogues, the *Thermus* species do not obey this rule. A

particularly exciting contribution of the thesis is the discovery of a distinct superinfection exclusion mechanism based on mini-CRISPR arrays encoded by two viruses infecting *Sulfolobus* archaea. The study of the abundance and nature of these mini-CRISPR array sequences indicate that these viruses compete for access to host cells and, once established, protect the cell from infection from the second virus. Notably, these data reveal a mechanism that contributes to speciation process in viruses. Finally, the thesis work revealed that Thaumarchaeal integrated mobile genetic elements also encode CRISPR arrays against other thaumarchaeal mobile elements thus further reinforcing the “guns for hire” concept.

Taken together, the presented thesis is of high scientific quality and novelty and thus constitutes an important contribution to the current state of the art in the field.

- The quality of publications

Sophia Medvedeva co-authored six original articles, two as first or co-first author. Five articles were published in peer reviewed international journals and one article was submitted. All articles were published in first quartile (top 25% of the impact factor distribution) journals for microbiology/ecology category, thus attesting the high quality of publications.

- The summary of issues to be addressed before the thesis defense

A minor issue that should be addressed before the defense concerns the general introduction chapter of the manuscript. Most of the figure legends in this chapter are incomplete and some are missing (exemple figure 5), this should be corrected before publication of the manuscript.

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