

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.

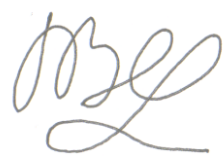
Supervisor: Professor Konstantin Severinov

Chair of PhD defense Jury: Professor Yuri Kotelevtsev

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Date of Thesis Defense: October 24, 2017

Name of Reviewer: Petr Sergiev

<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: 06-09-2017</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 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

CRISPR/Cas system is an adaptive immune system of bacteria and archaea. Molecular mechanism underlying the function of this system is of great interest to the scientific community due to the high value for the fundamental as well as applied research. The dissertation is nicely structured and clearly written. The literature review is informative and up to date. The questions addressed in the dissertation are related to the mechanisms of CRISPR functioning for two types of CRISPR/Cas systems, which were not yet investigated at sufficient details. Namely, I-F type CRISPR/Cas system from *P. aeruginosa* and particular *E. coli* strain was investigated, as well as type III-A and III-B systems from *Thermus thermophilus*. The methods chosen for the study perfectly match the goals. Apart from standard genetic manipulations with *E. coli*, genome and plasmid manipulations with *T. thermophilus* were done. In the course of the work several state-of-the-art results were obtained. It was demonstrated, that

1. In contrast to I-E, all components of CRISPR-Cas machinery are required for both naïve and primed adaptation in I-F subtype system.
2. The distribution of acquired spacers from the region surrounding the priming point in I-F system is opposite to the distribution observed in I-E system.
3. Interference but no adaptation was observed by *T. thermophilus* III-A and III-B CRISPR-Cas systems.
4. Multiple mismatches at the 3'- and 5'- ends of crRNA and the target have no effect on interference by III-A and III-B systems.
5. If the seed exists in III-A and III-B subtype systems, it has a complicated geometry and may be non-continuous.

Additionally, a very interesting observation was made that naïve adaptation for I-F system has a preference for plasmid DNA and particularly for the origin region. Unfortunately, the explanation of this fact is currently lacking.

The results are published in 2 peer-reviewed publications in high-impact scientific journals. I have no doubts that the thesis fits the best standards in the field.

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