

Jury Member Report – Doctor of Philosophy thesis.

Name of Candidate: Olga Musharova

PhD Program: Life Sciences

Title of Thesis: Investigation of DNA-binding specificity of Cas1-Cas2 CRISPR adaptation complex in *E.coli*.

Supervisor: Professor Konstantin Severinov

Chair of PhD defense Jury: Professor Philipp Khaitovich

Email: p.khaitovich@skoltech.ru

Date of Thesis Defense: October 17, 2017

Name of Reviewer: Vsevolod V. Belousov

I confirm the absence of any conflict of interest	Signature:  Date: 12-09-2017
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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

The dissertation of Olga Musharova “Investigation Of Dna-Binding Specificity Of The Cas1-Cas2 Crispr Adaptation Complex In *Escherichia Coli*” deals with one of the most recently discovered and intensively studied components of prokaryotic immune system, CRISPR-Cas. The thesis is of a very good quality, written clearly. The majority of the results are published in peer-reviewed journals.

More specifically:

The review of the literature is a good and sufficient introduction into the field that summarizes types of prokaryotic immune defence mechanisms and explains in details what is currently known about CRISPR-Cas systems: history of their discovery, its classes and types, components responsible for adaptation and interference. Separate chapter is devoted to application of CRISPR/Cas in biotechnology/biomedicine. The section could be better illustrated as such processes as CRISPR adaptation and interference are sometimes better understood from the schemes than from the text. Figure legend in Fig. 2 is missing. Although it is a scheme, it would be helpful to have a legend summarizing adaptation and interference processes.

The objectives of the project are clearly defined in the section following the literature review and mainly aimed on elucidating specific mechanisms of Cas1-Cas2 adaptation complex interaction with genomic and target DNA early in the CRISPR adaptation process.

Methods section is detailed enough to reproduce all the procedures used in the study. All the methods, from the basic DNA engineering and protein purification techniques to next-generation sequencing are absolutely relevant.

Results section is clearly written, each experiment is done in, at least, triplicates, and all the necessary controls are done. In particular, the most interesting results demonstrate that the Cas1-Cas2 complex interacts with the leader region of the CRISPR array during primed adaptation, that Cas1-Cas2 complex-associated DNA plasmid fragments are not double-stranded. Further the author demonstrates that CRISPR in process of CRISPR adaptation Cas3 nuclease converts fragments of the target DNA into a single-stranded form.

The discussion section summarizes the data obtained in the model of adaptation concerted by Cascade, Cas-3 and Cas 1&2 activities.

The dissertation topic is completely relevant to its content

The results obtained in the work bring important new details to our understanding of mechanisms of acquired prokaryotic immunity. The early events in the CRISPR adaptation cascade were largely unknown and this thesis provides new important insights into this process. The thesis content quality is further supported by four peer-reviewed publications, one is the Nucleic Acid Research paper with a first authorship. This paper describes the major set of the results appearing in the thesis and therefore reflects the main content of the dissertation.

Taken together, the quality of the work is very good and there are no serious issues to address before the defence.

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