

## 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](mailto:p.khaitovich@skoltech.ru)

**Date of Thesis Defense:** October 17, 2017

**Name of Reviewer:**

<p>I confirm the absence of any conflict of interest</p> <p>(Alternatively, Reviewer can formulate a possible conflict)</p>	<p><b>Signature:</b> <b>P. Sergiev</b></p>  <p><b>Date: 28-08-2017</b></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 spacer adaptation. This step is one of the least clear in the field. The methods chosen for the study perfectly match the goals. In the course of the work several state-of-the-art results were obtained. It was demonstrated, that

1. The Cas1-Cas2 complex interacts with the leader region of the CRISPR array during primed adaptation;
2. The Cas1-Cas2 complex is associated with spacer-sized fragments originated from a plasmid during primed adaptation and extent of association correlates with efficiency of adaptation of such fragments into CRISPR array;
3. Cas1-Cas2 complex-associated DNA plasmid fragments are not double-stranded.
4. Cas3 nuclease produces long single-stranded DNA fragments of target plasmid DNA during CRISPR interference and primed CRISPR adaptation;
5. Single-stranded breaks flanking hot protospacers occur in a non-target strand of plasmid from which primed adaptation occurs and require both Cas3 and the Cas1-Cas2 adaption complex.

The results are published in 4 peer-reviewed publications in high-impact scientific journals.

A model of spacer adaptation was suggested on the basis of the results obtained. The only point, that was not sufficiently clear is the mechanism of conversion of Cas1/Cas2 associated DNA from single stranded to double stranded form.

#### **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*