Skoltech
Skolkovo Institute of Science and Technology

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:

I confirm the absence of any conflict of interest (Alternatively, Reviewer can formulate a possible conflict)	**Signature:** **Date: 23-09-2017**

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

Bacteria and archaea acquire immunity to viruses and plasmids by integrating short fragments of foreign DNA into clustered regularly interspaced short palindromic repeats (CRISPRs). Our understanding of how these loci are used to generate short CRISPR-derived RNAs (crRNAs) that guide CRISPR-associated (Cas) nuclease to complementary targets has progress rapidly; however, mechanism(s) associated with new sequence selection and integration remain less well understood. Cas1 and Cas2 were shown to be necessary and sufficient in for naive sequence integration (i.e. initial vaccination) into E. coli (type I-E) CRISPR locus (Yosef *et al*), but escape mutants arise under selective pressure from the immune response. Mutant targets are detected by the crRNA-guided surveillance complex (Cascade), and trigger a "priming" response the involves all components of the I-E immune system. The work in this thesis aims to extend our previous understanding of adaption by determining the DNA specificity of the Cas1-Cas2 complex during primed CRISPR adaptation by the *E. coli* type I-E CRISPR-Cas system. Major findings include the accelerated rates of primed adaptation when Cas1 and Cas2 are overexpressed, that the repeat sequence is a strong self-signal that prevents primed adaptation, Cas1 and 2 interact with the "leader" in an IHF dependent fashion during primed adaptation, and Cas1-2 bind to protospacers that are not dsDNA. Aside from spelling and grammatical mistakes, the work is presented in a logical format. The questions addressed are of high-importance to the field and the experiments are well-conducted, but explanations of both the experimental design and results are sometimes difficult to follow.

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*