



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:

<p>I confirm the absence of any conflict of interest</p> <p>(Alternatively, Reviewer can formulate a possible conflict)</p>	<p>Prof. Udi Qimron Clinical Microbiology and Immunology Sackler School of Medicine Tel Aviv University</p> <p>10-09-2017 Date: DD-MM-YYYY</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 ✓

see attached document

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

September 10th 2017

Review of the PhD thesis of Ms. Olga Musharova

The PhD thesis submitted by Ms. Musharova focuses on studying the detailed mechanism of primed adaptation of the type I-E CRISPR system. For this purpose, she generated a valuable non-commercial reagent – anti-Cas1 polyclonal antibody. She cleverly used this antibody to uniquely show different stages of the adaptation process. She first showed that Cas1-2 interact with the leader sequence during primed adaptation. She further showed that Cas1-2 interact with specific pre-spacers. This is a significant achievement as it likely captures an intermediate step prior to spacer adaptation, an intermediate shown for the first time. Musharova also showed that the pre-spacer is probably not in a dsDNA form. Furthermore, she showed that Cas3 produces a ssDNA, which is presumably used for pre-spacer substrates. The work successfully accomplished its aims of revealing novel mechanistic insights on the adaptation process. This work paves the way for complete understanding of the adaptation process, which recently was shown to be harnessed as a molecular recorder for molecular events and even for digital images such as a picture or a movie.

The thesis is overall well written and thorough. The introduction nicely explains what the existing knowledge was, and emphasizes the gaps in knowledge. It explains the logic for carrying out the study, and provides the relevant background. Nevertheless, some parts in the Introduction are not relevant to the thesis, and more relevant to a thorough review (e.g. chapters on restriction enzymes, programmed cell death, etc.). The methods are presented clearly, with enough details to reproduce the results and to understand the workflow. The results and discussions are also presented clearly and logically. It summarizes all the data and relates it to existing knowledge. Significantly, the results of the thesis were published in a respectable journal – Nucleic Acids Research.

The topic of the dissertation fully matches the results presented in the thesis. The results are significant and contribute to the understanding of the adaptation mechanism. These results may facilitate development of applications relying on the adaptation step, such as molecular recording of molecular events.

Overall, the thesis submitted reflects significant achievements. I therefore recommend to recognize this thesis as fulfilling the requirements for granting a PhD degree.

Sincerely,

A handwritten signature in blue ink, reading "U. Qimron", is displayed on a light blue rectangular background. The signature is written in a cursive style with a horizontal line underneath the name.

Prof. Udi Qimron, Tel Aviv University