

## Jury Member Report – Doctor of Philosophy thesis.

**Name of Candidate:** Aleksandra Strotskaya

**PhD Program:** Life Sciences

**Title of Thesis:** Effects of Targeting by the *Esherichia coli* I-E CRISPR-Cas System on Infection by Various Phages.

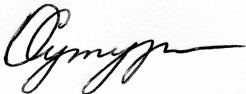
**Supervisor:** Professor Konstantin Severinov

**Chair of PhD defense Jury:** Professor Yuri Kotelevtsev

**Email:** [y.kotelevtsev@skoltech.ru](mailto:y.kotelevtsev@skoltech.ru)

**Date of Thesis Defense:** October 24, 2017

**Name of Reviewer:**

I confirm the absence of any conflict of interest	<b>Signature:</b>
(Alternatively, Reviewer can formulate a possible conflict)	
	<b>Date: 22-09-2017</b>

*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 work of Aleksandra Strotskaya, supervised by Pr. Konstantin Severinov, focus on the comparative analysis of the effects of targeting by the *Escherichia coli* CRISPR-Cas system on the infection by various bacteriophages. The manuscript of 127 pages is organized in 5 chapters including the introduction, the review of the literature, materials and methods, results and discussion and conclusions. The introduction section underlines the significance of the work, clearly states the objectives, explains the novelty and the practical use of this work and specifies the personal contribution of the candidate. The dissertation contains a large review of the literature on the bacteriophages, their diversity and life cycles, as well as defense strategies of bacteria to survive on phage infection. All steps of CRISPR-Cas system activity in bacteria, including CRISPR adaptation, crRNA biogenesis, and interference, are then described followed by the review of our knowledge on the interactions of phages with bacteria having CRISPR-Cas system. This introduction is complete and well structured, but lacks the illustrations. On my opinion the addition of figures describing different aspects of phages and CRISPR-Cas systems would be helpful for the readers. In general, I would suggest an additional round of proofreading to improve the quality of the manuscript. For example reformulating some long sentences would be helpful for the easier reading of the manuscript.

In the chapter 2, Aleksandra Strotskaya gives a general view on our current knowledge on the diversity of the bacteriophages and their life styles describing the steps of lytic phage infection including the phage absorption, the penetration of viral genetic material, metabolic changes inside infected bacterial cells, the assembly of phage particles and the host cell lysis. Different defense strategies of bacteria are then discussed that could interfere with phage development on each of these stages. The last part of this chapter is devoted to the CRISPR-Cas system functioning and gives the current state of art about this adaptive immunity system action.

The materials and methods section details the main experimental procedures used during this work.

The results and discussion section describes the main findings on the *E. coli* cells interactions with different lytic bacteriophages, i.e. M13, lambda, T5, T7, T4 and R1-37. The experimental strategy developed in this work for creation of a collection of *E. coli* strains targeting different genomic regions of phages was published in "Methods in Molecular Biology" with the participation of Aleksandra Strotskaya as a first author. Distinct consequences of infection in the presence of active CRISPR-Cas system were observed for cells infected with different phages. These results raise interesting questions on the action of this defense system during phage infection. The data represents detailed monitoring of the effects of type I-E *E. coli* CRISPR-Cas system action on the lytic DNA phages with different lifestyles. Interesting and poorly explored aspect of the work assess the timing of CRISPR-Cas defense during phage infection. The results show that CRISPR-Cas interference provides a strong selective pressure on diverse lytic phages that are able to rapidly overcome it by accumulation of escape mutations. The analysis with two phages with modified DNA, T4 and R1-37, demonstrates the ineffectiveness of the type I-E CRISPR-Cas system against these phages.

Finally, the major findings of the work are summarized in the last conclusions section. On my opinion, the perspectives of the work and future directions could be discussed in this part.

In conclusion, the results of Aleksandra Strotskaya represent an important scientific contribution to better understand the mechanisms of bacterial interactions with phages. Aleksandra Strotskaya signs an article published in international peer-reviewed journal "*Nucleic Acids Research*" in 2017 as the first author describing the action of type I-E CRISPR-Cas system on lytic bacteriophages. Many results presented in the dissertation are still need to be finalized, but should bring to the publication of additional articles. Aleksandra Strotskaya contributed as a first author for a method article published in "*Methods in Molecular Biology*" in 2015. She also participated

in an article on the primed spacer acquisition by type I-E CRISPR system published in a high-ranked journal "*Proceedings of the National Academy of Sciences*" in 2016 as a 4th author. For all these reasons I consider that the work of Aleksandra Strotskaya is sufficient for obtaining a Ph.D. degree of Skolkovo Institute of Science and Technology.

**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*