Skoltech
Skolkovo Institute of Science and Technology

Jury Member Report – Doctor of Philosophy thesis.

Name of Candidate: Anna Maikova

PhD Program: Life Sciences

Title of Thesis: The CRISPR-Cas system of human pathogen *Clostridium difficile*: function and regulation

Supervisors: Prof.r Konstantin Severinov;

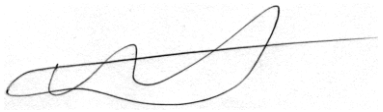
Prof. Olga Soutourina, University of Paris-Saclay, France

Chairmen of PhD defense Jury: Prof. Mikhail Gelfand, Skoltech ***Email:*** m.gelfand@skoltech.ru

Professor Harald Putzer, Paris Diderot University, France ***Email:*** putzer@ibpc.fr

Date of Thesis Defense: 30 September 2019

Name of the Reviewer: Dr. Mart Krupovic, Institut Pasteur, France

I confirm the absence of any conflict of interest	**Signature:**  **Date: 24-08-2019**

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 submit signed copy of the report 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 relevance of the topic of dissertation work to its actual content
- The relevance 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

In the present thesis, Anna Maikova presents the results of her PhD project focusing on the function and regulation of the CRISPR-Cas systems in a human pathogen *Clostridium difficile*. The PhD project has been carried out in the framework of a joint program between the Paris Diderot University, France and Skoltech, Russia.

The thesis manuscript is carefully conceived and clearly written. It consists of five Chapters. The first Chapter provides a thorough review of the literature pertinent to the thesis project. Parts of this Chapter have been published as a mini-review article in *Front Microbiol* with Anna as the first author. I really enjoyed reading the literature review, which sets the stage for the Results part.

The description of the Results starts with the second Chapter, which explores the functionality of the CRISPR-Cas systems in two different strains of *C. difficile*. The candidate has determined the protospacer adjacent motifs (PAM) and experimentally validated their functionality. The interference and naïve adaptation activities have been also confirmed for type I-B system of *C. difficile*. Finally, a deletion mutant of the full *cas* gene operon has been constructed and its interference activity assessed. The results showed that the remaining partial *cas* gene operon is sufficient to provide immunity, although it was less efficient that the wild type strain.

The third Chapter is dedicated to the study of the regulation of *C. difficile* CRISPR-Cas system and discovery of new type I toxin-antitoxin systems colocalizing with the CRISPR-Cas systems. It is shown that both CRISPR-Cas and toxin-antitoxin systems are coregulated and are under the control of the alternative sigma B factor associated with the general stress response. I find the latter discovery to be particularly interesting. The candidate also demonstrates the functionality of the toxin-antitoxin system and the ubiquity of associations between the toxin-antitoxin and CRISPR-Cas systems across more than 2000 sequenced *C. difficile* strains. This chapter also describes the potential regulation of one of the CRISPR arrays by a riboswitch. Although interesting in itself, the inclusion of this last part into Chapter 3 feels somewhat artificial and could be either relocated into Chapter 2 or presented as a separate chapter (I leave it up to the candidate to decide whether to react on this suggestion). Parts of Chapter 3 have been already published in *Nucleic Acids Research*, a high-profile peer-reviewed journal, with Anna as a co-first author.

Chapter 4 describes the successful attempts to harness the endogenous type I-B CRISPR-Cas system of *C. difficile* for genome engineering in this human pathogen. The corresponding manuscript has been submitted and is currently under revision. Finally, Chapter 5 provides general conclusions and future perspectives arising from the PhD project.

It is clear that during this work Anna has familiarized with a range of methods and molecular biology techniques. As can be judged from the above summary of the dissertation, during a relatively short time, the candidate has performed an impressive amount of work, which led to both new scientific discoveries and development of potential applications/tools. The presented results and the thesis manuscript itself are of high quality and meet the international standards for PhD thesis. Thus, I believe that Mrs. Anna Maikova deserves to defend her thesis in front of the jury and be awarded with the PhD degree.

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*