Skoltech
Skolkovo Institute of Science and Technology

Jury Member Report – Doctor of Philosophy thesis.

Name of Candidate: Elena Kurilovich

PhD Program: Life Sciences

Title of Thesis: The role of genome maintenance proteins in primed CRISPR adaptation by the type I-E CRISPR-Cas system

Supervisor: Professor Konstantin Severinov

Name of the Reviewer: Yuri Kotelevtsev

I confirm the absence of any conflict of interest (Alternatively, Reviewer can formulate a possible conflict)	**Date: 18-08-2023**

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 dissertation is well written in canonical way. It contains 134 pages, 36 Figures of which 29 expanded figures present major experimental results of the study. The literature review is well written and sets the state of the art mechanisms of CRISPR adaptation in Type I and Type I-E systems. It also gives review of genome maintenance systems, specifically Rec BCD and Rec FOR. The review logically leads to the aims and goals of the experimental study. The Materials and Methods section is written clearly and allows thorough reproduction of the results. Results are well presented and appropriately discussed. Conclusions are supported by the results.

- The relevance of the topic of dissertation work to its actual content

Dissertation is devoted to evaluation of yet unknown mechanism of conversion of exogenous phage DNA to spacers in CRISPR array during primed CRISPR adaptation. The defendant hypothesises envolvement of genome maintenance and DNA metabolism genes in this process. To support experimentally this hypotheisis the candidate genes were selected and their role in spacer acquisition.

The main result of the thesis support the hypothesis and establishes the essential role of role of *recJ* in CRISPR adaptation affecting prespacer generation and the accuracy of spacer incorporation. Double knockout of *recB* and *sbcD* revealed their essential role in primed adaptation. Moreover, the absence of RecBCD and SbcCD affect the pattern of acquired spacers in *Escherichia coli* type I-E CRISPR-Cas system. It allowed to underpin the initial hypothesis by showing contribution of the key genes of the genome stability maintenance to primed spacer acquisition. 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 thesis contains experimental results significant for understanding the mechanism of primer adaptation. It was demonstrated that whyle RecJ is essential, ExoVII encoded by *xseA* can replace the RecJ function in its absence. In double *recJ xseA* mutants the formation of adaptation intermediates is abolished. Double *recB recJ* mutants, were also dramatically compromised in CRISPR adaptation. This suggests the additive roles of RecB and RecJ in prespacer formation..

- The relevance of the obtained results to applications (if applicable)

P. aeruginosa is an important opportunistic human pathogen classified as a priority one pathogen by the World Health Organization. The pathogens were found to possess functional CRISPR-Cas systems, 30% of Type I-F and other 6% of Type I-E. Obtained results are highly relevant to the clinical practice, particularly they can be applied for the selection and engineering of phages which may be more efficient to due to breaching the defence of the host.

- The quality of publications

  Publications are of the highest quality with the top journal in the list Science Advances, with impact factor 14.

The summary of issues to be addressed before/during the thesis defense

Although the chapter 4.1 **Bacteriostatic antibiotics promote CRISPR-Cas adaptive immunity by enabling increased spacer acquisition** is methodologically linked to the thesis it is not contributing to the main subject, involvement of genome maintenance proteins in primer adaptation. I believe that the oral presentation will benefit if this chapter will be omitted.

Provisional Recommendation

X☐ *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*