Jury Member Report – Doctor of Philosophy thesis.
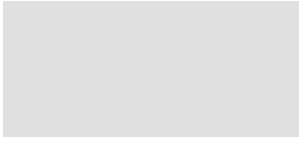
Name of Candidate: Elena Kurilovich

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: Assistant Professor Ekaterina Khrameeva

I confirm the absence of any conflict of interest (Alternatively, Reviewer can formulate a possible conflict)	 **Date: 05-09-2023**

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

- **Brief evaluation of the thesis quality and overall structure of the dissertation.**
  The thesis describes three studies. In the first one (Section 4.1), Elena Kurilovich and her colleagues found that the use of bacteriostatic antibiotics promotes the evolution of CRISPR immunity in bacteria. These antibiotics delay the production of mature phage particles during a phage infection. This delay provides more time for the bacteria to acquire spacers, which are important for CRISPR immunity. Therefore, in addition to factors like defective phages and nucleases that cleave phage genomes, the timing of phage development inside the infected cell plays a critical role in the acquisition of CRISPR immunity against the phage. In the second study (Section 4.2), authors examined how proteins responsible for maintaining genome stability contribute to spacer acquisition in the Escherichia coli type I-E CRISPR-Cas system targeting its own genome. In the third study (Sections 4.3 and 4.4), by applying FragSeq and biochemical approaches, authors show that RecJ is the main exonuclease trimming 5' ends of prespacer precursors.

- **The relevance of the topic of dissertation work to its actual content.**
  As the thesis investigates the links between genome maintenance proteins and CRISPR-Cas machinery, the topic of the thesis is relevant to its actual content.

- **The relevance of the methods used in the dissertation.**
  Methods used in the thesis are relevant and applied correctly, to my best knowledge, in both presented studies. The used methods are well described and presented with enough details, though I would suggest to expand the description of the sequencing data analysis part a bit, for clarification (see below).

- **The scientific significance of the results obtained and their compliance with the international level and current state of the art.**
  All three studies utilize state-of-the-art methods for studying CRISPR immunity and the role of genome maintenance proteins in this process. Thus, this thesis copes with the international level. The results described in the thesis are novel and interesting.

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

- **The quality of publications.**
  High enough to pass the PhD program requirements.

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

The thesis of Elena Kurilovich presents a high-quality work, both in research content and in writing. Therefore, I only have a few minor suggestions regarding the presentation of results in my area of expertise - sequencing data analysis and statistics.

1) Regarding the section 3.2.2:

   - It is unclear how reads containing two or more repeats were searched for. Was some specific software used for this task, or a custom script? I guess it is important to make sure that all such cases were accounted for, therefore these details matter.

   - About non-uniquely mapped spacers: did you check that they originate for rRNA operons? I could not find this analysis in the thesis.

   - About the normalization to the total spacer counts: did you also normalize for the library size (total number of reads)? Is it important to normalize for the coverage difference between samples?

   - It is ok to average results across two independent experiments but their high similarity has to be demonstrated first, to ensure reproducibility of results. This analysis of replicates consistency should be added to the thesis.

2) Page 74: "at the border of statistical significance" is more conventionally formulated as "marginally significant". Consider this replacement.

3) In Figure 27, Welch's t-test is used to assess the statistical significance of differences. However, this test requires normality of the data as one of its assumptions. Was it tested? I could not find it in the thesis. Either check the normality here an in all similar figures in the thesis or consider replacing with another test not requiring normality (Wilcoxon test).

The literature review presents a comprehensive description of existing knowledge on CRISPR immunity and genome maintenance proteins. It is very well written and contains all the details necessary for understanding of the research presented in next chapters. Other chapters are clearly written as well and I have no suggestions on possible improvements of their contents.

Provisional Recommendation
V *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