Skoltech
Skolkovo Institute of Science and Technology

Jury Member Report – Doctor of Philosophy thesis.

Name of Candidate: Anna Shiriaeva

PhD Program: Life Sciences

Title of Thesis: Interference and primed adaptation intermediates in type I CRISPR-Cas systems

Supervisor: Professor Konstantin Severinov, Skoltech

Name of the Reviewer: Konstantin Lukyanov

I confirm the absence of any conflict of interest	**Signature:**  **Date: 08-10-2020**

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 her dissertation work Anna Shiriaeva studied detailed molecular mechanisms of primed adaptation by the CRISPR-Cas systems of two types. The main focus of the research was on structure of prespacer DNA fragments generated *in vivo* in *E. coli* upon activation of CRISPR-Cas immunity against own genomic locus. The author developed FragSeq – a new method of high-throughput analysis of short DNA fragments (both single stranded and double stranded) generated in live *E. coli* cells. Comparative bioinformatics analysis of FragSeq results from different mutants and conditions allowed making solid conclusions about structure of the generated prespacers and their distribution in the genome. Moreover, Anna for the first time demonstrated direct involvement of some non-Cas enzymes into prespacer generation. FragSeq itself is an achievement as this method is applicable to a wide range of experimental models.

The thesis is written very clearly and logically. The literature review is broad and at the same time deep (more than 300 references). The Results section guides the reader step by step through the extensive experimental material obtained, carefully formulating and explaining the meanings of each established fact. Importantly, Anna clearly indicated all uncertain issues where additional studies are required. Conclusions are concise and fully supported by the results.

The major part of the results of dissertation was published in Nature Communications (IF=12.1, Nature Index Journal) with the first authorship of Anna Shiriaeva. Also, Anna is an author of a paper in Genes (IF=3.76), and the first author of a paper in Biochemical Society Transactions (IF= 5.16).

Overall, this is a top-level work, which is nearly ready for the formal thesis defense. I have only a few minor remarks and questions:

1. Fig.12A. In the Methods, this experiment is described as follows: “At various time points postinduction, cells were plated with serial dilutions on 1.5% LB agar plates for counting colony-forming units (CFU)”. At the same time, the curves in the panel are continuous and smooth; no experimental data points are shown. Please comment. Does it show average results of several independent experiments or one representative example? It should be described in the figure legend in more detail.

2. “To achieve this goal, we developed FragSeq – a protocol for strand-specific high-throughput sequencing of short (<700 nt) single-stranded and double-stranded fragments generated in vivo”. Why did you choose this length if the expected prespacer sizes are much less? Please add some explanation to the text for clarity. Did you check the size distribution in the obtained samples (e.g., by gel-electrophoresis)? If so, please show these data as a Figure.

3. Fig. 16B,C: Y axes are marked “% fragments per 1 kb” and the values are up to 0.1. Is this really 0.1%? If so, it corresponds to 10 b per 1 kb only. Is it sufficient to draw any solid conclusions? Or did you mean 10% (i.e. 0.1 with no “%”)? The same question is applicable to several other Figures showing similar experiments.

4. Is it possible from your FragSeq data to roughly calculate the number of prespacer molecules per cell accumulated during primed adaptation?

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*