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:**

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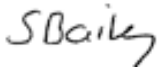
|---|---|
| I confirm the absence of any conflict of interest | **Signature:**  **Date: 09-10-2020** |

*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

The topic of this thesis is the CRISPR adaptive immune system in prokaryotes. Specifically, it deals with perhaps the least well-understood step in the building of CRISPR arrays, prespacer generation.

Overall the thesis is well written and beautifully illustrated. The literature review provides a clear and concise description of the CRISPR and DNA repair fields. The organization of chapters into project objectives, materials and methods, results and discussion is logical and flows well. The thesis is of very high quality.

A large component of the thesis is developing a new method, FraqSeq. This is a high-throughput sequencing approach (Illumina platform) for analyzing short DNA fragments present *in vivo*. The development of this new method is a significant technical achievement and provides a valuable tool for the CRISPR field and the field of DNA repair. Combining FraqSeq with genetics the candidate was able to bring new, and important, incite into how prespacers are generated in vivo.

The candidate found that prespacers are asymmetric double-stranded DNA fragments, matching spacers, with short 3 or 4-nt overhangs derived from excision of genomic DNA within a PAM. These prespacers are generated in a bidirectional, orientation-dependent manner relative to the targeted protospacer. Generation of prespacers required Cascade, Cas1, Cas2 and Cas3. In addition, at least two host enzymes, RecJ (a 5'-3' exonuclease) and the RecBC helicase are also required. These host enzymes were shown to be involved in the trimming of the 5'-ends.

These results shed light on a long-standing question in the CRISPR field and are highly significant. As such, the publications resulting from this work, Shiriaeva (2019), Kurilovich (2019) and Shiriaeva (2020) are high quality publications published in high impact journals with international reputations.

**Questions to discuss during the thesis meeting:**

1. How do you interpret the results of Xue et al (2016), conformational changes in the NTD of Cse1, with your model? Or more generally what role, if any, do you believe these conformation changes play in priming?

2. What is your hunch about the identity of nuclease X?

3. How does the length of the trimmed 5’-end compare with the structure of Cas1-Cas2? In other words, does Cas1 protection set the length of 5’ overhangs like it is believed to do with the 3’ overhangs.

4. What are your thoughts on 5’ overhang cleavage by cas1-cas2 reported by Radovcic et al (2018).

**Provisional Recommendation**

**X** *I recommend that the candidate should defend the thesis by means of a formal thesis defense*