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Skolkovo Institute of Science and Technology

Jury Member Report – Doctor of Philosophy thesis.

Name of Candidate: Andrey Krivoy

PhD Program: Life Sciences

Title of Thesis: Primed CRISPR-Cas adaptation in type I-E system of Escherichia coli: use of single-molecule and biochemical assays to verify models of the phenomenon at molecular level

Supervisor: Prof. Konstantin Severinov

Chair of PhD defense Jury: Prof. Konstantin Lukyanov ***Email**: k.lukyanov@skoltech.ru*

Date of Thesis Defense: November 30, 2018

Name of the Reviewer: Timofei Zatsepin

I confirm the absence of any conflict of interest (Alternatively, Reviewer can formulate a possible conflict)	**Signature:** **Date: 19-11-2018**

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

Doctoral Thesis by Andrey Krivoy is devoted to a hot topic of regulation of primed adaptation in type I-E CRISPR-Cas system.

Results are significant plus techniques developed or validated in the study open opportunities to answer crucial questions in the field in the future. Thorough description of materials and methods simplify further studies by followers. Study is solid; however some data in chapter 4.8 could be omitted. Andrey used very diverse methods: technical improvement of the device, magnetic tweezers, protein expression and in vitro assays - a rare story in Life Sciences. I want to emphasize that during this study Andrey successfully overcome numerous technical issues with magnetic tweezers (for example, temperature control). Obtained results would not lead to some applications in practice, but they support development of new instruments for basic research.

Major issues:

Review of the literature is only 12 pages, including the key part = models of CRISPR-Cas primed adaptation – only 1 (!) page. Thorough comparison of methods used in previously published papers on Cascade functioning will better explain the choice of magnetic tweezers as the main instrument of the study. This part would benefit from description of PAM identification by Cascade, assembly and translocation of CRISPR-Cas primed acquisition complex and conformational regulation of these processes. Also discussion of mechanistic driving forces for choosing between priming and interference will broaden the review of the area.

Discussion part will win from comparative analysis with published data in first part of 2018: 10.1128/mBio.02100-17, 10.1016/j.celrep.2018.01.045, etc.

Paper in NAR is very good. The publication in Methods in Enzymology does not fit Skoltech guidelines from a formal viewpoint - MiE is not a peer-reviewed journal. This is a book of protocols that is only edited to fit the journal get-up with a proofreading by a native speaker if needed.

Minor issues: Synthetic genes discussed in the text are not genes, they are synthetic DNA constructs. p.29 – biotin is not sensitive to freezing/thawing. Proofreading of the thesis should be done to improve the quality.

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*