

Jury Member Report – Doctor of Philosophy thesis.


Name of Candidate: Iana Fedorova

PhD Program: Life Sciences

Title of Thesis: Characterization and application of CRISPR-Cas enzymes

Supervisor: Professor Konstantin Severinov

Name of the Reviewer:

<p>I confirm the absence of any conflict of interest</p> <p>(Alternatively, Reviewer can formulate a possible conflict)</p>	<p>Signature:</p>  <p>Date: 03-09-2020</p>
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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.

It was my pleasure to assess the PhD thesis of Iana Fedorova . The thesis is written in a non canonical way and consists of a literature review followed by compendium of PDF files of six excellent published papers, including Cell and Nature communications and Nature Biotechnology where Iana made significant contribution. One paper in NAR has Iana as a joint first author and most recent paper in RNA biology has Iana as a joint corresponding author. This last paper was conceived by Iana and executed excellently. If NAR paper "DNA Targeting by Clostridium Cellulolyticum CRISPR-Cas9 Type II-C System" is technical characterization of PAM sequence and crRNA of a new member of class II Cas9 proteins, the second one describes novel finding of cut position dependence from the length of the spacer length of the guide RNA. This clearly indicates dynamic

model of Cas9 interaction with the target and requires further investigation which may produce even more exciting results. The literature review can be published as a perfect up to date description of different classes of Cas proteins, methods of their discovery using bioinformatics algorithms and experimental platforms for biomedical applications.

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

The topic of the dissertation was determined by the main scientific thrust of Professor Konstantyin Severinov laboratory on finding short and efficient variant of Cas protein for genome modification using AAV vectors. The thesis represents functional characterization, PAM sequence and crRNA determination of several Type II-C Cas9 orthologues: PpCas9, CcCas9. In the studies studies of Type V-A CRISPR-Cas systems effector Cas12 (former Cpf1 it was mapped the Cas12a cut site positions. It was found that these enzymes cleave DNA in a highly similar manner and that the length of DNA overhangs generated by Cas12e can be increased using guide RNAs with shorter spacer segments. Also IAn participated in the uncovering of the mechanism of acquisition of new spacers into the CRISPR array based on original HTS-based method FragSeq. The content of the dissertation follows to the point the topic and the established goals.

- The relevance of the methods used in the dissertation

The thesis capitalizes on the state of the art several laboratories in Moscow, SpB and Boston. All used methods were immaculately executed with rational design of multiple sets of conclusive experiments.

- The scientific significance of the results obtained and their compliance with the international level and current state of the art

The results were published in the best scientific journals passing the most stringent review which demonstrate the highest international level and significance of the results

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

Thesis lists several already awarded and under review patents.

- The quality of publications

All Q1 publications including Nature series and Cell

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

In conclusion, Iana Fedorova joined as a junior member a serious study conducted in leading laboratories aimed at identification and characterisation of new effective tools for genome editing. She contributed significantly in several projects at the earlier stages, produce first author paper and then, finally managed to accomplish her own study being the corresponding author. This is a spectacular progress for a PhD student and I recommend this thesis to be accepted as it is and award Iana Fedorova with PhD.

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