

Jury Member Report – Doctor of Philosophy thesis.

Name of Candidate: Anna Maikova

PhD Program: Life Sciences

Title of Thesis: The CRISPR-Cas system of human pathogen *Clostridium difficile*: function and regulation

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Date of Thesis Defense: 30 September 2019

Name of the Reviewer:

<p>I confirm the absence of any conflict of interest</p> 	<p>Signature:</p> <p>Date: 22-08-2019</p>
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Reviewer's Report

The topic of the thesis clearly is interesting. While the general properties of the CRISPR-Cas seem to be reasonably well understood (although surprises are still possible), the diversity of these systems has not been assessed in any depth. At that, each particular system is likely to bring some novelty, and indeed, one of the main results of the presented doctoral research is co-localization and likely co-regulation of one of the *C. difficile* systems with an active toxin-antitoxin module, and elucidation of the properties of the latter. Similarly, each well-characterized CRISPR-Cas system may yield something of practical importance, and, based on her research, the author has created a CRISPR-Cas genome editing system for *C. difficile* and demonstrate that it outperforms existing genome manipulation approaches for this medically important bacterium.

The review chapter is detailed and comprehensive, demonstrating the candidate's perfect command of the area and good writing skills. Indeed, one of the candidate's published papers is a review.

The results of research are strong and solid, and the applied methods are relevant and up to date. Some results have been already published in a good journal, and there is no doubt that the remaining ones conform to strictest international standards and hence are likely to be published in near future.

Still, I have some remaining comments.

- Section 2.3.1.2 “The consensus sequences of selected PAMs were then visualized by the WebLogo tool (Crooks et al., 2004). This analysis demonstrated that the -4 position of the PAM does not play any role in *C. difficile* CRISPR-Cas system functioning (data not shown).” — Visual analysis of Web-logo is not sufficient to prove that a position is not relevant, statistical analysis of the distribution at that position as compared to non-PAM positions in the same context is required.
- Figure 2.3. There are clear differences in the PAM frequencies in two considered strains (A and B);the given explanation “Different patterns of CCN and TCN PAMs distribution in 630 Δ erm and R202091 strains could be a consequence of the different amount of good-quality selected reads in the libraries “after”.” Is somewhat superficial: why would filtering of reads affect positional nucleotide frequencies in PAMs?
- Section 3.3.1.4 — many of identified ORFs could be spurious, clustered due to residual sequence conservation in genomes of closely related strains. It might be a good idea to analyze positions of nucleotide polymorphisms in the candidate genes relative to the reading phase.

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