

Jury Member Report – Doctor of Philosophy thesis.

Name of Candidate: Viktor Mamontov

PhD Program: Life Sciences

Title of Thesis: Escape mechanisms of mobile genetic elements against CRISPR-Cas system and diversity in microbial communities

Supervisor: Professor Konstantin Severinov

Name of the Reviewer: Simon Jackson

I confirm the absence of any conflict of interest	
(Alternatively, Reviewer can formulate a possible conflict)	Date: 30-12-2023

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

- Brief evaluation of the thesis quality and overall structure of the dissertation.

Overall, the thesis is generally of a suitable quality but could be improved (mainly introduction, as below). However, large parts of the thesis (Chapters 3 and 4) are not the candidate's own work. Chapter 2 (primarily the candidate's work) is of high quality. In terms of structure and content, I am not aware of the expectations of the host institution. At my own institution we generally expect 2-3 'solid' results chapters which are primarily the candidate's own work. In this case, Chapter 2 meets the standard but Chapters 3 and 4, seem a little 'thin' for a thesis by the standards at my institution.

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

The introduction (Chapter 1) and first results (Chapter 2) are relevant to each other. The other two chapters seem less relevant to the central thesis.

- The relevance of the methods used in the dissertation

The methods are appropriate.

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

Chapter 2 clearly meets the required standard. The scientific advances made by the candidate in Chapters 3 and 4 are less clear.

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

N/A

- The quality of publications

Chapter 2 is a high-quality publication that makes a valuable contribution to the field.

General comments on each Chapter:

Chapter 1 introduces the thesis topic (focused mainly toward the work presented in Chapter 2). In general, this section is adequate. However, some sections are a little disjointed, jumping between sentences/ideas and in general the section lacks 'polish'. E.g. p18 "... conjugative plasmids can replace the host TA system with a more..." To which TA system are you referring? P23 "... K-12 strain harbors an induced type I-E system..." It should be made clear that this is an engineered variant where the Ara/IPTG inducible promoters have been inserted upstream of the *cas* genes. In places, some key references are lacking. E.g. Swarts et al 2012 for priming.

Chapter 2 is clearly the highlight of the thesis and details a substantial investigation into the plasmid interference activity of a CRISPR-Cas system in an engineered *E. coli* system and how plasmids can persist in a sub-population of cells despite CRISPR-Cas targeting. The experiments are well-planned and executed, with clear rationale for each experiment, sound interpretation and subsequent design of follow-on experiments. The work has been peer reviewed and published in a reputable journal. The

candidate is the first author of the manuscript and appears to have performed the majority of the work, aside from the mathematical modelling. The work makes a valuable contribution to the field and the candidate should be commended for their efforts. In addition, Chapter 2 contains unpublished data that extend the findings of the study to look at M13 phage persistence when targeted by the *E. coli* type I-E CRISPR-Cas system.

For chapter 3, it is difficult to assess the candidate's caliber as a researcher from this chapter as the work is relatively mechanical (not allowing for a great deal of scientific enquiry) and the candidate's contributions (as presented) are relatively short. It appears the candidate's direct contributions comprise the data and writeup presented in pages 87 to 94. It is unclear whether the Discussion (3.4) is solely the candidate's work, or that of other authors. For the bioinformatic analyses presented, the candidate states (p12) that they were responsible for the analyses of the 16S data. However, the methods list a github account belonging to someone else for the 16S data analysis pipeline (p77). The github repository listed is not publicly available, so I am unable to further assess this. It will be necessary for the candidate to clarify their own direct contributions to the analyses. Overall, whilst the resulting manuscript (once peer-reviewed, revised and published) will be useful from a technical perspective (e.g. for future metagenome studies), the chapter in the thesis does not substantially demonstrate the candidate's abilities to formulate hypotheses, design, perform and interpret experiments, discuss findings in relation to existing knowledge etc. Some opportunities to include more in-depth discussions have been overlooked, for example shotgun metagenomic approaches are not mentioned, except in the introduction. What is the rationale for pursuing 16S alone and not also short or long read shotgun sequencing in parallel (given the limitations of 16S highlighted in the introduction)?

For Chapter 4 (where the candidate is listed as 5th author), the candidate's contributions are stated as "all technical aspects" and "substantial contributions to the in silico analysis of the sequenced strains...". Similar to Chapter 3, the relatively technical and short nature of the candidate's contributions limit assessment of the candidate's research skills. The author contribution statement (p13) is a little vague, "... I made substantial contributions to the in silico analyses...". It is not clear whether this means the candidate performed the analyses presented in 4.2.1.1, .2, .3, or .4 (and which parts were performed by or alongside other authors).

Chapter 5 (Conclusions):

Consider moving the new data (Fig. 5.1 and supplementary figures) to the results Chapter 2. Some sentences/parts of the Conclusions chapter are rather vague, for example "... are related to the studies of diversity in microbial communities." Some 'claims' of new/advances are rather incremental (at best), for example "Our results illustrate the efficiency of bioinformatic approaches to search for valuable biosynthetic gene clusters based on long-read sequencing technology." Was a comparison made to genome assemblies generated by short-read sequencing? And "The potential of long-read sequencing and in silico analysis for the study of new bacteria isolates was demonstrated." There are myriad previous publications demonstrating use of long-read sequencing for bacterial genome assemblies and analyses, so this does not seem a substantial advancement of the work.

Final remarks:

The candidate is clearly capable of high-quality independent research (Chapter 2) and has demonstrated some additional technical skills (bioinformatics analyses) in Chapters 3 and 4.

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