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Jury Member Report – Doctor of Philosophy thesis.

Name of Candidate: Matvei Kolesnik

PhD Program: Life Sciences

Title of Thesis: RNA targeting defence systems in prokaryotes

Supervisor: Professor Konstantin Severinov

Name of the Reviewer: Andrey V. Kulbachinskiy

I confirm the absence of any conflict of interest	**Date: 11-12-2024**

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

The thesis written by Matvei Kolesnik is dedicated to RNA-targeting CRISPR-Cas systems, focusing on the Type VI CRISPR-Cas system from *Leptotrichia shahii* and the Type III CRISPR-Cas system from *Thermus thermophilus*. Type III CRISPR-Cas systems are diverse, highly complex, and still underexplored despite a long history of study. Type VI CRISPR-Cas systems, which are strictly RNA-specific, are of particular interest due to their collateral RNase activity, responsible for the cleavage of host transcripts. In addition, there are chapters dedicated to analysis of unusual RNA polymerases found in some bacterial viruses and to isolation of novel phages infecting *Thermus thermophilus.*

The thesis is presented as a collection of seven published papers. The first paper investigates the diversity of CRISPR spacers in *Thermus* bacteria from environmental hot springs. Among its results, the observed presence of locally specific Type III CRISPR spacers suggests that the Type III CRISPR-Cas system actively acquires new spacers from viral genomes. The paper provided in Chapter II describes the reconstitution of the spacer acquisition process in Type III CRISPR arrays of *T. thermophilus* in laboratory, enabling the investigation of the mechanisms governing spacer selection.

Paper provided in Chapter III is a project focused on the functions of phage-encoded virionic RNA polymerase. Chapter IV provides a comprehensive literature review of the biology of Type III CRISPR-Cas systems. Within the work provided in Chapter V, Matvei investigates the role of auxiliary RNase effectors in the immune response mediated by Type III CRISPR-Cas systems in *T. thermophilus*. The results demonstrate that the auxiliary Type III-associated RNases of *T. thermophilus* are conditionally essential for immune responses against plasmids.

Chapter VI provides an exciting discovery of the role of collateral RNase activity in the immune response mediated by Type VI CRISPR-Cas system from *L. shahii*. The findings reveal that *L. shahii* Cas13 effector protein works as anticodon nuclease cleaving tRNA molecules upon the recognition of foreign transcripts, leading to the inhibition of protein synthesis. These results are intriguingly connected with the recent discoveries of tRNA-targeting prokaryotic immune systems and with the fact that many bacteriophages encode their own tRNAs which are presumably expressed in order to compensate for the degradation of cellular tRNAs.

The final chapter describes an isolation and analysis of several newly identified bacteriophages infecting *T. thermophilus*. While not directly aligned with the thesis's primary focus, this work provides interesting insights, particularly regarding phage-encoded defense systems.

The methodologies selected for the research are well-elaborated and appropriate for addressing the research questions. The study makes valuable contributions to the understanding of prokaryotic defense systems, particularly Type VI CRISPR-Cas systems from *L. shahii*. The results have been published in high-profile journals, including *Nature* and *Science Advances*.

Critical comments and suggestions:

1) Although each of the chapters has indubitable scientific interest and was published in reputable (and in some cases top-ranked) journals, their connections with each other are not always clear. Each chapter should be placed in the context of the whole work by adding at least a brief description of how it relates to the rest of the thesis. This analysis should be placed at the beginning of each chapter.
2) Furthermore, the author should try to put all the parts of the thesis together in the Conclusions section. Currently, it contains several paragraphs separately summarizing the findings of individual chapters, even missing some of them. As stated in the Skoltech's PhD Thesis Defense Policy, the thesis based on the work associated with the peer-reviewed academic papers should be accompanied by appropriate explanatory, connective, and integrative analysis, and should not be a mere compilation of published papers themselves.
3) I also recommend summarizing the main findings of the study as several short bullet points at the end of the thesis, similar to conclusions usually found in traditional 'candidate of science' theses.
4) The author's contributions should be comprehensively outlined at the beginning of each chapter.
5) Chapter VII contains a BioRxiv preprint version instead of the final article published in Viruses, this should be replaced.

I believe that the required corrections can be successfully introduced in the final thesis text before the defense. In conclusion, the project represents a significant body of work and undoubtedly deserves to be defended as a PhD thesis at Skoltech.

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*