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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: Konstantin Miroshnikov

I confirm the absence of any conflict of interest	**Date: 06-12-2024**

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

Dissertational manuscript by Matvei Kolesnik titled " RNA targeting defense systems in prokaryotes" describes the studies of molecular details of Type III and Type VI CRISPR-Cas systems which may be unified as "RNA-targeting prokaryotic defense systems". These systems serve as an important part of a bacterial counteraction against specific bacteriophages (bacterial viruses). Spacer acquisition process and target requirements in the course of Type III CRISPR-Cas functioning in *Thermus thermophilus* demonstrated the efficacy of the system cells against bacteriophages with DNA genomes. Also, the products of RNA degradation were studied for *Leptotrichia shahii* Type VI CRISPR-Cas system. Additionally, the activity of virionic DNA-dependent RNA polymerase of CrAss-like phage phi14:2 demonstrated interesting links between early transcription in phage infection and gene silencing process.

The dissertation contains a lot of novel scientific data. Many aspects of fine details of particular CRISPR-Cas systems were first revealed in the course of the experiments and first described in the reviewed dissertation. The work done illustrates the diversity of mechanisms the prokaryotes use to protect themselves from viral infection. The novelty of the performed research makes the scientific value of Matvei Kolesnik's dissertation undisputable.

Theoretical analysis of the diversity of bacterial Type III CRISPR-Cas protection mechanisms was published as a review in Biochemistry (Moscow), the leading Russian journal in the area. Experimental results were published as original articles in a high-impact journals (Nature, Nucleic Acids Research, Journal of Molecular biology). The results were also discussed as verbal and poster presentations at the scientific conferences. The representation of the results completely fulfils the requirements to the doctoral dissertation.

The results are presented and analyzed clearly and convincingly. No major issues are formulated through the practical part of the dissertation. The only critical note can be expressed: Each part of the dissertational manuscript presented as an article reprint, is comprehensive. However, the general idea bringing the overall experiments together, is not obvious. This may be a shortcoming of the used format of the dissertation and hopefully, it will be clarified in the course of the verbal presentation. Nevertheless, this issue does not depreciate the value of the work done.

In my opinion, the dissertational manuscript by Matvei Kolesnik contains very important work that is of great interest to the research community. The introduction and conclusions are well written. The results of each chapter are sound and convincing. Overall, the author of the thesis presented, Matvei Kolesnik, deserves to be awarded the doctoral degree.

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*