

Jury Member Report – Doctor of Philosophy thesis.

Name of Candidate: Karyna Karneyeva

PhD Program: Life Sciences

Title of Thesis: Exploring type III CRISPR-Cas immunity in *thermus thermophilus*

Supervisor: Professor Konstantin Severinov

Name of the Reviewer: Mikhail S. Gelfand

I confirm the absence of any conflict of interest

Date: 18-03-2024

Reviewer's Report

The review chapter is well written, although too laconic in parts. In particular, given the importance of distinction between suicidal and restrictive defense systems, a more detailed review of their variety might be beneficial (in particular, the author does not mention toxin-antitoxin systems that were one of the first discovered suicidal systems). Similarly, non-defense functions of CRISPR-Cas systems deserve more attention. On the other hand, the review of Type III CRISPR-Cas systems, most relevant to the thesis, is quite extensive, which is good.

Methods are diverse, relevant and describe in sufficient detail. The author mentions the site of proteomic analysis (para. 3.19) and some sequencing analysis (para. 3.12, but without data on the fragment and read lengths), but not other sequencing rounds (para 3.13; 3.14, no detail; 3.16 and 3.17, Oxford Nanopore - where?).

Chapters 4 and 5 are preceded by acknowledgements of the colleagues' help with the bioinformatics, proteomics, and modeling analysis; these notes should be more detailed (what analyses have been performed by whom?). This may be addressed during the defense.

Overall, chapters 4 and 5, mainly based on published papers, are well written, the described results are interesting, important, and convincing. The exposition is clear, and graphical illustrations useful and elegant.

The Conclusions are clear, although a bulleted list might be useful to understand what particular results the author selects as the most important ones.

Overall, the thesis is strong and sufficient for a defense. Publication requirements have been met, with three papers in journals with IF>2, one

of them being a first-author paper. The results have been presented in two good international conferences, that again satisfies the requirements.

Still, I have a number of editorial comments.

Chapter 1 "Introduction", 1st paragraph: "The sophisticated relationships between prokaryotes and MGEs have intrigued researchers for over a century" – indeed? Who was that genius who had been intrigued by MGEs back in 1924?

Page 80: 50% phages in supernatants of collapsed cultures contained intact protospacers. Does that mean that phages with intact protospacers still could overcome CRISPR-Cas and proliferate?

Page 83, the last paragraph: is faster elongation rate of the phage RNA polymerase an established fact or a conjecture? In the former case a reference should be provided; in the latter case it might be better to reformulate in softer terms.

Page 115: "We assume that the free energy of binding of a pair of complementary nucleotides ΔG is additive and identical for all complementary base pairs" – how valid is this assumption? Is it crucial in the sense that the modeling results are robust with regards to the value of ΔG ?

Page 122: "Our findings demonstrate the effectiveness of Type III-A and III-B CRISPR-Cas systems in protecting *T. thermophilus* cells from phiFa and phiKo phage infections solely" – I'm not sure I understand this sentence. In what sense "solely"?

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~~