Skoltech
Skolkovo Institute of Science and Technology

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:

I confirm the absence of any conflict of interest (Alternatively, Reviewer can formulate a possible conflict)	 **Date: 16-03-2024**

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

Reviewers report should contain the following items:

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

The dissertation is well written in canonical way.

The dissertation is well written in canonical way. It contains 143 pages, 36 Figures of which 34(including 6 supplementary) figures present major experimental results of the study. The literature review is well written and sets the state of the art life cycle strategies of bacteriophages and bacterial defence mechanisms with sprcific aspect on Type III CRISPR-Cas systems, layers of complexity, structural basis : requirements for target recognition and most interesting, and relevant to the thesis experimental part, the strategies of phage escape of bacterial immune system.
The review logically leads to the aims and goals of the experimental study. The Materials and Methods section is written clearly and allows thorough reproduction of the results. Results are well presented and appropriately discussed. Conclusions are supported by the results. The Materials and Methods section is written clearly and allows thorough reproduction of the results. Results are well presented and appropriately discussed. Conclusions are supported by the results.

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

The central topic of the dissertation is related to the evaluation of the mechanisms used by detect phages to escape bacterial immune response. It was demonstrated that genomes of these phages accumulated deletions of definite number of codons in essential target genes and complete deletions of non-essential genes. The author defined the target requirements for both Type III-A and III-B systems for model plasmid transformation interference. Using two variants of the vector plasmid, each carrying a protospacer matching a spacer from the genomic CRISPR array of T. thermophilus. This allowed to identified the minimal continuous length of the target-crRNA heteroduplex necessary for plasmid transcript recognition, arresting bacterial cell growth on selective medium. Author hypothesis that Type III effectors bind targets through a simple bimolecular reaction, compensating lower target abundance with more extensive crRNA-target base pairing.

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- The relevance of the methods used in the dissertation

The methods used in the dissertation are adequate.

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

The dissertation contains a number of novel scientific results. Particularly, it was demonstrated that the minimal RNA duplex sufficient for interference can occupy multiple positions within the Type III-A effector. The study defined the crucial role of HD nuclease domain of the Cas10 effector subunit for efficient Type III-A immunity.
Taken together, this study widens our knowledge about the interaction of mobile genetic elements with co-occurring Type III-A and III-B CRISPR-Cas immunity of T. thermophilus.

- The relevance of the obtained results to applications (if applicable)
  These findings are of pure scientific nature, however they might be useful in construction of therapeutic phages for fighting antibiotic resistant infections.
- The quality of publications
  The publications are all relevant to the topic of the dissertation and are in leading journals Nucleic Acids Res IF 14,9 , Journal of Molecular Biology IF 5,6

The summary of issues to be addressed before/during the thesis defense

I have no issues to be addressed before/during thesis defense

Provisional Recommendation

X☐ *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*