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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)	**Edze Westra** **Date: 18-03-2024**

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 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

This is a well structured and well executed thesis that aims to improve our understanding of the mechanism and function of Type III CRISPR-Cas immune systems (III-A and III-B), using the thermophile Thermus thermophilus as a model organism.

CRISPR-Cas are immune systems that confer phage resistance in a sequence specific manner through the integration of phage-derived sequences (known as spacers) into CRISPR loci on the bacterial genome. Transcripts of these CRISPR sequences are processed and used by CRISPR-associated proteins (Cas proteins) to detect and destroy complementary nucleic acids, or to trigger alternative (usually cell dormancy or death) responses. While the process of spacer acquisition and interference is well understood for some CRISPR-Cas systems (e.g. Type I and II systems), Type III systems remain comparatively less well studied / understood.

Chapter 1 and 2 provide a thorough introduction / literature review and a description of the methods used in the thesis. Chapters 3 and 4 are the two experimental chapters of this thesis, that examine key aspects of Type III CRISPR_-Cas mediated resistance and escape from resistance.

In the first experimental chapter, they generate Thermus thermophilus clones carrying mini-CRISPR arrays that encode spacers against phiKo and phiFa phages. These clones are used to monitor resistance phenotypes against phage infection, and how these patterns depend on the spacer sequence and position of the target on the phage genome (and whether target genes are expressed early/mid/late during infection), and whether resistance is mediated by the Type III-A or III-B system. They also selected and characterized phage mutants that evaded the Type III CRISPR-Cas immune response, identifying large deletions in the phage genome that enable these mutants to escape CRISPR immunity.

Next, they use a plasmid transformation model to study in more detail the mechanism of target recognition by the Type III effectors. They analyse what mutations enable bacteria to associate with the plasmids and identify the role of between CRISPR-array recombination events. Next, they analyse in detail the tolerance of the III-A and III-B CRISPR-Cas systems to mismatches between the spacer and the target sequence, and the role of base pairing between crRNA repeat sequences and the target-flanking sequence in self/non-self discrimination. Differences in tolerance of III-A and III-B systems to mismatches are explained by differences in immune complex abundances as well as differences between the effector proteins of each immune system – specifically the HD nuclease domain that is present in III-A systems and not in III-B systems.

Overall, the thesis comprises a comprehensive piece of work that is of sufficient quality and rigour to proceed towards public defense. There are many interesting elements that are worthy of discussion – I intend to focus on the coevolutionary consequences of the observed phenotypes associated with the III-A and III-B systems, how this may depend on environmental parameters, and to what extent observed phenotypes may depend on the presence of alternative defence systems in the bacterial genome.

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*