

## 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:** Raymond H.J. Staals

I confirm the absence of any conflict of interest



**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

The PhD thesis of the candidate describes an in-depth study of the type III CRISPR-Cas system of *T. thermophilus*, a thermophilic bacterium. The study is subdivided into 6 chapters in a logical order: starting with an introduction chapter that underlines the importance of studying these so-called anti-viral defense systems in prokaryotes, followed by a more zoomed-in perspective of the main topic of the thesis (type III systems), by exploring the literature in Chapter 2. Chapters 3, 4 and 5 describe the actual experimental work done by the candidate, where the underlying procedures are outlined in detail in Chapter 3. The candidate has applied a healthy mixture of *in vivo* and *in vitro* experiments using (mainly) well-established techniques that are very appropriate to address the research questions the candidate was trying to answer. The last chapter is a recap of all the new obtained knowledge and placing the candidate's findings into the bigger picture. As such, the content of the thesis is very much in agreement with the topic.

The thesis was also a pleasure to read due to the abovementioned structuring, but also in terms of the uncomplicated writing style and the careful formatting of the figures, making it a very digestible piece of work, even for a non-specialized reader.

The obtained results described in the thesis have led to new insights into these complex immune systems, that will most likely spark new research in the field. The work of the candidate has made an important contribution to the international community that studies these systems. What really makes this work stand out over other studies, is the use of the native host (where these type III systems are encoded). Whereas most labs use heterologous overexpression systems (mostly in *E. coli*), the candidate opted to perform many of the experiments in *T. thermophilus* and its related phages, making the obtained data very unique and valuable. I think the thesis mostly contains new insights that are relevant from a fundamental biology point-of-view. However, certain aspects of the thesis will also be useful for more applied science, as demonstrated by the use of type III engineer phage genomes, described in Chapter 4.

The importance of this thesis is also reflected and acknowledged by the high quality of the peer-reviewed and internationally-recognized journals where these works were published: a very recent publication about the target RNA requirements in the Journal of Molecular Biology, a review on type III CRISPR-Cas biology in Biochemistry (Moscow) and a study on the type III spacer acquisition preferences (from phage) published in Nucleic Acids Research. The latter two works have already been cited quite a few times (20 and 24 citations respectively).

Taken together, I think the candidate has uncovered several important aspects about the modus operandi of type III CRISPR-Cas systems and also found some discrepancies with prior research works that will fuel future studies into these fascinating defense systems. As such, I have no further issues that need to be addressed prior to the defense.

#### **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*