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Jury Member Report – Doctor of Philosophy thesis.

Name of Candidate: Julia Gordeeva

PhD Program: Life Sciences

Title of Thesis: Recognition strategies of Type I and Type V BREX systems

Supervisor: Professor Konstantin Severinov

Name of the Reviewer: David Bikard

I confirm the absence of any conflict of interest (Alternatively, Reviewer can formulate a possible conflict)	**Signature:** **Date: 29/07/2022**

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

The thesis manuscript presented by Julia Gordeeva gathers works on the anti-phage defense system known as BREX. A conundrum that all immune systems face by definition is the differentiation between self and non-self. In the well described case of restriction methylation systems, this is achieved by the differential recognition of epigenetic methylation marks on the host and foreign DNA. BREX systems were discovered relatively recently and their mechanism of action as well as the mechanism of self versus non-self recognition is still poorly understood. This is the scientific problem that Julia Gordeeva tackles in her PhD manuscript.

Julia Gordeeva first offers an introduction to anti-phage defense systems in Chapter 1, with a particular focus on systems that modify DNA. These include restriction modification systems, DISARM, DPD, Dnd, Pgl and of course the BREX system which is the focus of the work presented. Other types of defense systems, CRISPR-Cas, abortive infection systems (including TA, retrons etc.), CBASS and more, are also presented, giving a well written and complete overview of the current knowledge in a fast-changing field.

Chapter 2 presents the materials and methods used during the experimental work, while the results are presented in Chapter 3. In a series of well-designed experiments taking advantage of the lysogenic cycle of phage lambda, Julia Gordeeva elegantly demonstrates that BREX modifies the DNA it recognizes as self, while restricting un-modified DNA. Further work on mutants of the BREX system revealed that all components are important for defense except for BrxA. While BREX shares some of the characteristics of RM systems, the mechanism of action seems to be quite different, with foreign DNA being degraded *in vivo* but not *in vitro*.

These results are followed by a series of experiment on a different BREX system from H. hispanica, that nicely corroborates the results obtained in *E. coli* while providing important insights into a different class of BREX systems and identifying the methylation marks.

Altogether the research methodology is appropriate and the manuscript well written. The interesting results gathered by Julia Gordeeva greatly contribute to our understanding of BREX systems and open new research directions. With this work, Julia Gordeeva demonstrates a strong expertise in bacterial genetics, a solid knowledge of the literature in her field and her ability to conduct rigourous research. I therefore believe that she deserve the grade of PhD and should defend her thesis.

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*