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Skolkovo Institute of Science and Technology

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 T.F. Dryden

I confirm the absence of any conflict of interest	**Date: 10-08-2022**

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

This thesis comprises three full chapters and a short discussion chapter plus a bibliography and appendices. In my opinion the thesis meets the general criteria listed above including a high quality publication in a highly respected journal.

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

However, the thesis does require the addition of some further material that the candidate has not seen fit to include though the experiments and information have undoubtedly been collected by the candidate.

I will list these corrections below. In addition many of the figures are rather small and, if possible, it would be desirable to increase their size.

The introductory chapter adequately describes the general area of restriction of phage propagation in prokaryotes and describes the current state of knowledge of classical restriction systems through to the most recent discoveries. The only thing missing from this introduction are the chemical structures of the most common nucleotide modifications such as N6-methyladenine. These structures would be useful for later in the thesis when the modification of cytosine by the H. hispanica BREX system is described.

The second chapter on materials and methods is rather brief for it to form a basis for another experimenter to reproduce this work. The accession numbers and full sequences for the BREX loci studied in the thesis need to be given. The sequences could be placed in an appendix with genes colour coded as in the sketches of, for example, plasmid pBREXAL. This would also allow promoter sequences and any overlaps between genes to be clearly shown, for example, the overlap between brxA and brxB is mentioned but not shown. In addition the domains and catalytic motifs mentioned in various sections could be marked.

This chapter, and in many locations throughout the remainder of the thesis, contains minor errors that should be corrected.

When bacteria are named, the species and the strain should be given every time they are mentioned, for example, just saying MG1655 is not sufficient. Similarly the phage lambda is often deployed but sometimes it is the wild type phage and other times it is a variant. However the text often does not distinguish between different phage. It would be a simple matter to make it clear on each occasion what strain and phage are being used rather than having to refer back to the methods section.

Correctly speaking, “bacteria are transformed with DNA” rather than having “DNA transformed into a bacterial strain”. Similarly DNA is “ligated into a vector” rather than “cloned into a vector”.

The third chapter describes the results obtained on the BREX systems of E. coli and H. hispanica. The experiments are well performed and give clear conclusions.

However, more data could be presented. For example, the efficiency of plating is often just quoted without any photograph of the spot tests or the titer of phage. These data should be shown. At other points the phrase “data not shown” is used. In my opinion a thesis should show all relevant data so the candidate should show this or delete the sections in question.

The pacbio data could be expanded upon and further analysed. The nature of the cytosine modifications found in the H. hispanica system is not given! I presume it is N4-cytosine rather than C5-cytosine but this is vital information. Further bioinformatics analysis of the Target Recognition Domains in the BREX methyltransferases relative to their DNA targets and to other similar methyltransferases in RM systems should be performed (the BREX methyltransferases show great similarity to the type IIG RM enzymes which also recognise asymmetric targets).

A further point that could be considered is the orientation and distance between successive BREX targets on phage and bacterial DNA as it is recognised that this is important for the functioning of some restriction systems.

The H. hispanica NucS protein has not been compared to the BrxU type IV restriction endonucleases – this would be easy to do.

The BrxL and BrxHII proteins are clearly important for the BREX restriction activity but no clear mechanisms have been given. The annotations of possible function differ between these two proteins suggesting that the annotations may be incorrect. It would be nice to read a more extended discussion of these proteins and the mechanism of restriction.

The attempt to show the activity of the BREX complexes in vitro unfortunately did not work, figure 20. I could not find a clear description of the methods used in chapter 2. It is possible that the cell extract preparation procedure inadvertently removed a key cofactor required for DNA degradation. It would be nice to read more about this experiment and some suggestions about the nature of the chemical degradation of DNA by BREX systems.

The thesis concludes with a short discussion and conclusions chapter. Perhaps the comments above would be useful in expanding the discussion further?

The appendices contain oligonucleotide sequences using the DNA manipulations. It would be a good idea to change the font to "Courier" as this makes the sequences all line up neatly. As mentioned above I wish to see the annotated sequences of the BREX loci as an additional appendix.

The bibliography is comprehensive in its coverage of the published literature and generally well formatted. However, many of the references lack full page numbers and some others are slightly inconsistent in format. The bibliography should be thoroughly checked for accuracy.

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*