

## Jury Member Report – Doctor of Philosophy thesis.


**Name of Candidate:** Aleksandra Galitsyna

**PhD Program:** Life Sciences

**Title of Thesis:** Chromatin folding in individual cells

**Supervisor:** Professor Mikhail Gelfand

**Name of the Reviewer:** Veniamin Fishman

I confirm the absence of any conflict of interest	 <b>Date: 14-10-2021</b>
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*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 “CHROMATIN FOLDING IN INDIVIDUAL CELLS” by Alexandra Galitsyna is dedicated to the development of novel computational methods for single-cell and population (bulk) Hi-C analysis and application of these methods to study principles underlying animals’ genome organization.

The thesis consists of nine chapters. The first, second, and third chapters give a brief overview of the current state of the 3D-genomic field and provide the thesis objectives. Although the introduction is brief, the text is well illustrated (in fact, almost every page contains an illustration), consciously written, and includes citations of the most recent literature, which allows the reader to quickly ingest the most important statements.

Each of the chapters four to eight describes a different research problem addressed by Alexandra. The largest part of the obtained results describes chromatin folding in individual cells, in agreement with the topic of the dissertation. Other studies also belong to the field of 3D-genomics; however, it seems that they are not always directly connected to the former part focused on single-cell genomics. This is not an objection; in contrast, it is commendable that Alexandra was able to explore different aspects of chromatin biology during her Ph.D. studies. In addition, Alexandra made an effort to explain a scientific logic connecting these projects, providing a short introductory text preceding the main results of each chapter.

The main results of chapters four to eight are also briefly summarized in the introductory text mentioned above, and next described in the details in the attached scientific papers. I enjoyed this style of presentation; however, sometimes it was difficult to distinguish the author’s contribution. For example, chapter five (“A machine learning framework for the prediction of chromatin folding in *Drosophila* using epigenetic features”) is based on the paper where Alexandra is one of the two co-first authors with equal contribution. I was not able to access Alexandra’s contribution to this work, neither from the manuscript text nor from the thesis. Note that this comment should be treated as an editorial suggestion rather than an essential concern about the author’s contribution, because in the majority of cases, including the part describing single-cell Hi-C data analysis, Alexandra’s contribution is clearly evident.

Finally, the last chapter in the thesis briefly summarizes the results obtained by Alexandra.

I would like to acknowledge the scientific contribution of Alexandra’s work to the growing field of single-cell genomics. Whereas single-cell RNA-seq is a relatively mature area, other single-cell techniques, including scHi-C, are only developing. Exploring such a new field is always exciting, but also requires careful analysis to distinguish technical artifacts from true biological effects. To this aim, Alexandra developed and implemented multiples approaches, metrics, algorithms, and standards, which will be important for future studies. For example, the ORBITA tool allows more accurate identification of Hi-C contacts in scHi-C data than previously published bulk Hi-C methods, and I expect that ORBITA will be used by other groups for scHi-C analysis in the future.

Using the methods of scHi-C analysis developed by Alexandra, her colleagues and herself were able to describe genome folding in individual *Drosophila* cells, which answers a very relevant and debatable question. This work brings new fundamental insights into chromatin functioning and dynamics.

In accord with the high methodological and scientific level of her work, Alexandra has a good publication record, including three publications where Alexandra is a first or co-first author.

Finally, I would like to give a few small editorial recommendations which Alexandra could address during the thesis defense:

1. In Fig. 2-1, it seems that 30-nm fiber is schematically shown. It is now believed that a 30-nm fiber does not exist in the majority of cells under physiological conditions (doi: 10.4161/epi.28297), thus Fig. 2-1 should be revised.
2. In Fig. 4-1, it is not clear what are white spaces between filled segments. One may speculate that these are inter-TAD regions; however, it is not obvious, because many TAD-callers call TADs as adjustment intervals not allowing inter-TADs. I would suggest explaining what these white spaces mean explicitly.

**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*~~