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Jury Member Report – Doctor of Philosophy thesis.

Name of Candidate: Aleksei Mironov

PhD Program: Life Sciences

Title of Thesis: Tissue-specificity and regulation of aberrant alternative splicing

Supervisor: Assistant Professor Dmitri Pervouchine

Name of the Reviewer: Dr Maria Poptsova

I confirm the absence of any conflict of interest (Alternatively, Reviewer can formulate a possible conflict)	**Date: 03-09-2022**

Reviewer's Report
The Doctoral Thesis of Alexei Mironov is devoted to the subject of alternative splicing, which is an important area of research. Specifically, Alexey studied the aberrant splicing (AS), the phenomenon that was poorly characterized till recently and development of the methods of AS detection, finding major factors associated with this phenomenon is of great importance. From the presented Thesis on can conclude that a great amount of work was performed by the author. The results of his finding were published in high-ranking journals such as Nature Communications and PLoS Computational Biology, that supports the value and importance of the conducted research. The presented Thesis is well-structures and well-written. It is framed in 8 Chapters. Chapter 1 introduces the area of research and specifies the subject of the dissertation, explaining why two types of aberrant alternative splicing - tandem alternative splicing sites (TASS) and unproductive splicing events (USE) - are chosen as the subjects of the research. In Chapter 2 the author presents the comprehensive background of splicing, explains its molecular mechanisms, describes known types. Alexei introduces the phenomenon of aberrant splicing and two AS types – TASS and USEs. Chapter 3 is a short statement of the Thesis research objectives that are focused on identification of tissue-specific aberrant splicing events - specifically TASS and USEs, and understanding the mechanisms of their regulation.

Chapter 4 is the detailed description of Methods including the identification of splice sites from annotation databases, expression splice sites from GTEx Consortium data, and prediction of cryptic splice sites. It describes the criteria of identification of TASS clusters, major and minor splice sites. It introduces the metric to quantitatively characterize tissue-specific usage of aberrant splicing sites and quantification of aberrant splicing sites. It also includes methods of exploring RBP effects and proteomics analysis as well as methods to study tissue-specific expression.

Chapters 5 and 6 present the main results of the Thesis. Chapter 5 describes how the most complete set of TASS was assembled by the author from known databases and based on the methods developed by the author. Then the author explores how the TASS evens are distributed over tissues, affect expression and protein structure, regulation by RBP, as well as he investigates TASS evolutionary patterns. The author identified a significant proportion of de novo TASS sites, especially minor splice sites. He introduces major (maSS) and minor (miSS) splice sites. Analysis of relative expression of miSS with respect to maSS in different tissues revealed prevalence of brain tissue, specifically cerebellum. In order to identify regulatory factors of TASS, the author identified tissue-specific miSS pairs that also differentially expressed upon RNA-binding protein (RBP) inactivation that lead to 256 miSS-RBP-tissue triples. Also, the analysis was done on the proteomic level, and author demonstrated the cases when miSS are included in protein, often in disordered region. Several case-study examples are provided. The chapter ends with the evidence for TASS evolutionary selection.

Chapter 6 is devoted to unproductive splicing. The author described how the USEs were assembled, and as it was the case with the TASS the author detected numerous USEs de novo. Then the author describes the analysis of tissue specificity and present cases of of validated tissue-specific USEs. The analysis of regulated USEs that bind RBP revealed tissue-specific clusters in brain, skeletal muscle, and heart. The chapter ends by the predicted network of regulated USEs with CLIP support for RBP-binding in the gene.

In the Discussion - Chapter 7 - the author discusses the possible mechanisms of regulation of TASS and USEs.

The Thesis ends by Conclusions that are presented as summary of main results of the research.

Overall the text is clearly written and easy to follow, however here is I list my comments and suggestions to the presented text:

To me the subject of tissue-specificity should be discussed in more details. For TASS sites this discussion is almost lacking. How the author explains relative abundance of AS events in one tissue compare to the other. Which tissue are more prone to aberrant splicing? The reader would benefit from obtaining the comprehensive view.

RBP analysis revealed some significant players associated with AS. It would also be beneficial to the reader if the author would summarize classes/types of RBP participating in all studied types of AS and highlight the corresponding pathways.

It would be also beneficial to present GO-enrichment analyses for genes detected as having AS events for TASS and USEs, both overall non-tissue-specific and tissue-specific.

But all these comments do not dimmish the value of the presented research which was done at high scientific level. To my opinion Alexei Mironov deserves the award of PhD degree.

Dr. Maria Poptsova

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Associate Professor
Head of the International Laboratory of Bioinformatics
Faculty of Computer Science
Big Data and Information Retrieval School
Higher School of Economics
11 Pokrovsky boulvar, Room T907
Moscow, 101000
Tel: +7 (495) 531-00-00 * 27335

https://cs.hse.ru/en/big-data/bioinform/
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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*