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Poznań Doctoral School of
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This is a review of PhD thesis titled "Genomic characterisation of long-noncoding RNAs in the zebrafish genome" written by Monika Kwiatkowska. The thesis is written in English and contains all necessary parts.

The objectives are clearly stated. The methods are carefully described in sufficient detail to allow others to reproduce them. The introduction is a good compromise between deep and specialised description and a general overview of the subject. The schematics included in the introduction are very well selected, correspond to the ideas described in the text and guide the reader through the concepts described.

The result section is divided into three parts that correspond to different stages of experimental and bioinformatic analysis performed. The result sections contain enough information to follow the flow of logic behind individual experiments. As a wet lab scientist, I especially appreciate the description of the bioinformatic pipelines and methods used.

In general, the thesis is well written with clear logic and only minor spelling mistakes. I would only like to highlight the inconsistency in the Figures panel labelling with some labelled in A, B and some as A), B) as well as an amusing inversion of A letter in Figure 4.20

In general, I find the results obtained compelling and well-described. I do, however, have several questions:

Figure 4.15 shows an analysis of Spike-ins sequencing results using CapTrap-seq and CapTrap-seq SS500. The author claims a slight improvement in the detection of longer RNAs. I am not sure I agree. It is clear that even if the improvement is present, the amount of undetected RNAs is also increasing. Additionally, it would be interesting to present the data normalised for the depth of each sequencing run.

The above "slight" or no improvement in a longer spike in RNA detection using the CapTrap-seq SS500 contrasts with the increase in the length of the transcript models derived from the sequencing results. Could the author comment on this? Given the small or no change observed with the spike in detection, could the length increase be attributed to the lower level of ribosomal RNA present in the CapTrap-seq SS500 libraries?

I also have a more general question that I would like the author to address. One of the aims of the thesis was to improve the annotation of lncRNAs in the zebrafish genome. I do appreciate that lncRNA annotation is an important goal, after all, I am studying lncRNA, and

annotation is often the starting point. But I wonder, given the DANIO-CODE data for CAGE (TSS) and 3'-seq, how the full-length lncRNA annotation will help experimental scientists. I guess, in the end, it comes down to the question of the presumed mode of action of a majority of lncRNA. If we presume it is the RNA, then full-length annotation is important, but if we assume it is the act of transcription, then having start and end sites may be enough. But maybe I am wrong. I would like the PhD candidate to discuss this point during the defence.

The submitted doctoral dissertation meets the requirements specified in the Act of July 20, 2018, Law on Higher Education and Science (Journal of Laws of 2018, item 1668 with amendments) as well as in the Procedure for Granting a Doctoral Degree at the Institute of Bioorganic Chemistry, Polish Academy of Sciences in Poznań (resolution of the Scientific Council of ICHB PAN no. 28/2024/Internet of March 20, 2024), and I request the Scientific Council of the Institute of Bioorganic Chemistry, Polish Academy of Sciences to admit Monika Kwiatkowska to the next stages of the procedure for awarding the doctoral degree.

Przedstawiona do recenzji rozprawa doktorska spełnia warunki określone w Ustawie z dnia 20 lipca 2018 roku prawo o szkolnictwie wyższym i nauce (Dz.U. z 2018 r. poz. 1668 ze zm.) oraz w Sposobie postępowania w sprawie nadania stopnia doktora w Instytucie Chemii Bioorganicznej PAN w Poznaniu (uchwała Rady Naukowej ICHB PAN nr 28/2024/Internet z dnia 20 marca 2024 r.) i wnioskuję do Rady Naukowej Instytutu Chemii Bioorganicznej PAN o dopuszczenie Moniki Kwiatkowskiej do dalszych etapów postępowania o nadanie stopnia doktora."

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