

Brno, May 20, 2020

PHD THESIS EVALUATION REPORT

Title: Methodology and application of quantitative microRNA analysis Applicant: Ing. Peter Androvič

University: Palacky University Olomouc, Faculty of Science,

Field: Experimental Biology

Assessment of the thesis:

The topic of thesis is original, current and relevant in the context of up-to-date research in the field of microRNAs (miRNA) with a strong potential in a wide range of biomedical applications. There were four main aims addressed in PhD thesis: (i) to develop, optimize and validate new method for miRNA quantification based on RTqPCR that would allow precise and cost-effective quantification from various samples including animal and plant tissues, cells and biofluids, (ii) to develop easy-to use tool that would allow convenient optimization and troubleshooting of the wet-lab workflow of quantitative miRNA studies as well as routine control of sample quality, (iii) to comprehensively evaluate all currently available methods for small RNA-Seq library preparation and (iv) to dissect global gene and miRNA expression changes after ischemic stroke and spinal cord injury (SCI) to reveal underlying molecular and cellular mechanisms.

For all these principal aims, Peter Androvič provided original data significantly contributing to the field. Despite the novelty of the topic, he reached interesting results, contributed to the development of new two-tailed RT-qPCR method, developed a quality control panel, performed sophisticated benchmarking of commercially available methods for small RNA-seq library preparation and finally described transcriptional response of young and aged mouse brain to ischemic stroke and SCI.

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Formally, the thesis is written on 53 pages including an extensive list of the bibliography (267 references). The content is divided into Introduction, Aims and scope, Literature review, Material and Methods, Survey of results, and Conclusions. Literature review provides a very useful and current overview of the study focus including miRNA genomics and biology, quantitative miRNA profiling, and roles of miRNAs in central nervous system. This well-written and concise introduction provides evidence of the deep theoretical knowledge and very good orientation of the author in the problem discussed in the thesis. All experimental methods used in this study are described in Materials and methods section in sufficient detail. Experiments and measurements are well summarized and valuable results are presented and discussed in a very concise and logical way in Survey of results referencing Figures and Tables from the concrete publications which are part of the thesis as a Supplements. The aims of the thesis were completed, methods of research work are appropriate and in agreement with the aims formulated in the thesis. The whole thesis is compact piece of work, containing five full-text papers as a supplement. In general, the thesis fulfils the formal requests at a very good level and I have no formal remarks.

Important aspect of Peter Androvič thesis is the fact that almost all data involved in the thesis were published in peer-reviewed international journals with impact factor. Author showed very good creative abilities and his publishing activity is highly above standards. He published 2 original papers as first author (Nucleic Acids Research, Scientific Reports), 1 review paper (Molecular Aspects of Medicine) and 2 original papers are submitted, currently as preprints in BioRxiv. Further, he is co-author of another 6 original papers on the topic related to PhD thesis.

Regarding the concrete scientific achievements, Peter Androvič,

- developed a novel RT-qPCR method called "Two-tailed RT-qPCR" for accurate measurement of miRNA expression in cells, tissues and biofluids,
- 2) established a quality control protocol allowing monitoring of technical workflow of miRNA expression studies,

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- performed a benchmarking study of library preparation methods with focus on biofluid samples and understanding of technical biases affecting the small RNA-Seq studies,
- provided a detailed insights into the impact of stroke aging and their interaction on genome-wide expression profiles.

Peter Androvič was trained and successfully used several up-to-date laboratory methods, especially in the field of molecular genetics, gene expression profiling and advanced bioinformatics. This thesis convincingly documented that he acquired necessary theoretical knowledge and practical skills and that he can create a working hypothesis and test it with appropriate series of experimental methods. Thus, he showed capability for independent scientific work and very good ability of creative work.

I have two questions for the author related to the PhD work:

1) What is your view on the stability aspects of circulating miRNAs (what is their half-time, how they are degraded, what is affecting their stability, is their stability sequence-dependent)?

2) What do you see as the optimal and the most reproducible method for cell-free microRNAs profiling in the body fluids and samples with extremely low RNA levels (independently on the cost)?

3) What is your view on the methodical aspects of isomiRs detection? What should be the optimal workflow for independent validation of the body fluid isomiRs detected in a small RNAseq experiment?

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Overall, quality of this PhD thesis is high above average. Author showed all necessary practical skills, theoretical knowledge and high motivation for research work. Quality of this thesis is fully in agreement with the common requirements for PhD degree.

Therefore, after successful defence of the PhD thesis, I recommend it to be accepted and doctoral degree be awarded to Peter Androvič.

Sincerely,

Chy Ma

Ondrej Slaby, Ph.D. Professor, Research Group Leader Central European Institute of Technology (CEITEC)

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