

Dr. Vladimír Beneš

Charles University in Prague
Faculty of Science
Department of Student Affairs
Albertov 6
CZ-128 43 Prague 2
Czech Republic

Review of Ph.D. thesis “Single cell gene expression profiling and quality control“ submitted for defence by David Švec, M.Sc.

Analyses of expression of genes in single cells, the lead project David Švec, M.Sc. has decided to work on during his Ph.D. studies is timely and of utmost relevance. Undoubtedly, any advance in characterization of processes ongoing in an individual cell contributes ultimately to our by far incomplete understanding of principles governing functions of whole organisms. However, even cataloguing the single cell content is still technically challenging and certainly not trivial. Moreover, to obtain adequate data sets enabling inference of correlations among individual functional components is methodologically even more demanding.

David carried out his Ph.D. studies, whose results are summarized in the submitted Ph.D. thesis, jointly at the Institute of Biotechnology, Czech Academy of Sciences, in Prague and at the TATAA Biocenter in Gotheburg, Sweden under supervision of Prof. Mikael Kubista. During his Ph.D. studies David primarily addressed these topics:

- development and implementation of protocols enabling controlled, stable and quantitative isolation of total RNA from samples with a low cell count including single cells;
- development and implementation of tools required for quality and quantity checks of low input RNA samples; this effort included optimization of tools for data analysis;
- application of these tools in analyses of gene expression in single cells;
- enhancement of previous developments of the high-resolution qPCR tomography to study distribution of mRNA transcripts in single cells at the sub-cellular level; David successfully applied his findings in analyses of *Xenopus* oocytes;
- enhancement of the qPCR tomography method by microscopic imaging to better characterize studied tissues and facilitate preparation of a temporal-spatial maps of distribution of mRNA transcripts there; David used this set-up to study dynamics of a murine molar embryonic development. This part has already yielded some promising results and is still in progress.

In pursuit of his Ph.D. project's goals David, being aware of limitations of standard procedures when applied to analyses of single cells, has conceived and successfully implemented several protocols as well as their improvements, including tools enabling him their robust control. In addition, he exploited series of complex molecular biology methods and high-end techniques, such as laser-capture micro-dissection, high-throughput microfluidics qPCR and others. His effort culminated in the very recent and valuable article published generously in the open-access journal *Frontiers in ONCOLOGY*. This article is a solid proof of David's achieved prowess and competence.

It is the Literature Review part of David's Ph.D. thesis I am rather uncomfortable with. Firstly, I dare to say that – and I emphasize – unfortunately, Czech language is generally not optimal for the topic presented in the submitted Ph.D. thesis. Highly technical English terms used in

the presented field transformed into Czech lead to awkward grammatical constructions (and David's Ph.D thesis is unfortunately no exception), causing that it's not only a reader but occasionally also the author himself who are lost... Regrettably, also due to this fact David's text frequently lacks flow and coherence, description of several methods is reduced beyond clarity, formal and factual errors notwithstanding (I can provide the list). Also David's use of jargon is well above the level customary for this type of document. Last but not least, despite of the fact that a Ph.D. thesis is not usually assessed as a composition essay I would appreciate that David had spent time and paid much higher attention to editing and proofreading of his thesis than he probably did. Let me put it this way: major stylistic revision would be required... Quality of the Literature Review part is in the stark contrast with considerably better quality of the text in attached publications. Is it perhaps because these were longer in making?

Overall assessment: Regardless of my comments, which concern rather dissatisfying style of the submitted text, I trust David's Ph.D. thesis meets current formal requirements for such a document. Considering David's achievements, which are convincingly documented by included and other publications he has co-authored, I also trust that he has accomplished goals set at the beginning of his Ph.D. studies.

I deem his Ph.D. thesis suitable for defence and recommend accepting it. Assuming his success at this act I am convinced that David has shown qualities required from a successful Ph.D. student. With that and with submitting his Ph.D. thesis along with an accompanying abstract booklet I believe David Švec, M.Sc. has fulfilled all conditions demanded for completion of Ph.D. studies.

Questions for a Ph.D. thesis' defence:

- How has the concept of a gene evolved in the light of recent findings? Should one distinguish between a transcript and a gene?
- Is an assumption that dPCR enables determination of truly absolute values of number of specific RNA transcripts truly valid? Absolute number of cDNA molecules, which indeed can be determined, derived from a particular RNA transcript need not at all correlate with number of RNA molecules of the original transcript. What are the caveats?
- Which class of transcripts can confuse and mislead a researcher in single cell analyses?
- Is it really necessary to use high-capacity methods for studies of single cells?
- How feasible is analysis of a single-cell epigenome and how one can approach it?



Dr. Vladimír Beneš

Heidelberg, 7 January 2014