Přírodovědecká fakulta
Faculty of Science

Jihočeská univerzita v Českých Budějovicích
University of South Bohemia in České Budějovice

OPPONENT'S REVIEW ON BACHELOR THESIS

Name of the student: **Stefanie Pezelj**

Thesis title: Generating a fluorescently tagged MARK2 fusion protein as marker for the basolateral membrane of preimplantation stage mouse emrbyo blastomeres
Supervisor: **Alexnader Bruce, Ph.D.**

Referee: RNDr. Alena Krejci, Ph.D.
Referee`s affiliation: **University of South Bohemia, Faculty of Science**

	Point scale[1]	Points
(1) FORMAL REQUIREMENTS		
Extent of the thesis (for bachelor theses min. 18 pages, for masters theses min. 25 pages), **balanced length of the thesis parts** (recommended length of the theoretical part is max. 1/3 of the total length), **logical structure of the thesis**	0-3	3
Quality of the theoretical part (review) (number and relevancy of the references, recency of the references)	0-3	3
Accuracy in citing of the references (presence of uncited sources, uniform style of the references, use of correct journal titles and abbreviations)	0-3	3
Graphic layout of the text and of the figures/tables	0-3	3
Quality of the annotation	0-3	2
Language and stylistics, complying with the valid terminology	0-3	3
Accuracy and completeness of figures/tables legends (clarity without reading the rest of the text, explanation of the symbols and labeling, indication of the units)	0-3	2
Formal requirements – points in total		19
(2) PRACTICAL REQUIREMENTS		
Clarity and fulfillment of the aims	0-3	3
Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions	0-3	3
Discussion quality – interpretation of the results and their discussion with the literature (absence of discussion with the literature is not acceptable)	0-3	3
Logic in the course of the experimental work	0-3	3
Completeness of the description of the used techniques	0-3	3

Experimental difficulty of the thesis, independence in experimental work	0-3	3
Quality of experimental data presentation	0-3	3
The use of up-to-date techniques	0-3	3
Contribution of the thesis to the knowledge in the field and possibility to publish the results (after eventual supplementary experiments)	0-3	3
Practical requirements – points in total		27
POINTS IN TOTAL (MAX/AWARDED)	46	(0-48)

Comments of the reviewer on the student and the thesis:

In her bachelor thesis, Stefanie Pezelj aimed to clone and test an expression construct for the MARK2 fusion protein, as a tool to mark the basolateral membrane of the preimplantation mouse embryo for future experiments. This was a nice and well defined project well suited for the Linz students that have rather limited time to work in the lab but at the same they want to learn as much of molecular biology as possible.

The thesis is very well written. The introduction is brief but focused, with minimal grammatical inaccuracies. The method section contains detailed description of the cloning procedure as well as the in vitro transcription and embryo injection protocol, documenting the realtively large scope of experimental procedure that Stefanie was exposed to. Nevertheles, some of the potentially useful information have been omitted, like the size of the pRN3-Venus vector and its more detailed map, the protocol for bacterial glycerol stock preparation or the actual structure of the Mark2 gene and its cDNA. The Result section documents the sequential steps during the cloning procedure and in vitro mRNA preparation as well as the actual fluorescent signal that localizes the MARK2 protein in the basolateral membrane of the mouse embryo, proving that Stefanie's project was overall successful. The gel pictures are sometimes a little bit confusing because they are overexposed and the sizes of the ladder bands are not labelled directly in the pictures but only in the legend description (and sometimes incorrectly like in Fig. 11 where the lowest visible band is certainly not 0.5kb). I would also expect to show the aligment of the cloned construct after sequencing.

I appreciate the precisely written Discussion that mentions the alternative strategies to improve the quality of the Mark2-Venus fluorescence signal and the protein stability, as well as the potential wider use of the Mark2 construct for experiments in the embryo. In fact, many of the questions that I intended to ask when reading the thesis were already answered in the Discussion, leaving me empty handed. This clearly shows that Stefanie understood the project and she was able to criticaly think about its wider significance and future directions.

In summary, I enjoyed reading Stefanies thesis and I consider it a very successful and nicely writen up project. With no hesitation I suggest the grade excellent.

Suggestions and questions, to which the student has to answer during the defense. Mistakes, which the students should avoid in the future:

1. You cloned the Mark2 cDNA into the pRN3-Venus vector using a single restriction site. Could you suggest a different cloning approach that would allow the insert to be always inserted in the correct orientation (and you do not have to check for the insert orientation after the cloning) ?

2. In Figure 10 you can see very weak bands of the correct size in the PCR screening of colonies that however do not contain the plasmid with correct insert. How could you explain the appearance of these bands in the PCR reaction?

3. You generated in vitro transcribed mRNA with and without the poly-A tail and you say both of these mRNAs were used for microinjections but then only the embryos injected with mRNA without the tail are shown. Why? Are there any other ways how to improve the stability of mRNA except the poly-A tailing?

Conclusion:

In conclusion, I

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the thesis for the defense and I suggest the grade excellent .

In **Ceske Budejovice** date **5th June 2019**

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signature