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: Stina Eßmann

Thesis title: Subcellular localization analysis of proteins non univocally located to the mitochondria of *Trypanosoma brucei*

Supervisor: Ignacio Durante, Ph.D.

Referee: RNDr. Zdeněk Franta, Ph.D.
Referee`s affiliation: Univerisity of South Bohemia, Institute of Chemistry

	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	2,5
Quality of the theoretical part (review) (number and relevancy of the references, recency of the references)	0-3	2
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	1,5
Quality of the annotation	0-3	2
Language and stylistics, complying with the valid terminology	0-3	1
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	1
Formal requirements – points in total		13
(2) PRACTICAL REQUIREMENTS		
Clarity and fulfillment of the aims	0-3	1,5
Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions	0-3	1
Discussion quality – interpretation of the results and their discussion with the literature (absence of discussion with the literature is not acceptable)	0-3	1
Logic in the course of the experimental work	0-3	1,5

[1] Mark as: 0-unsatisfactory, 1-satisfactory, 2-average, 3-excellent.

Completeness of the description of the used techniques	0-3	0,5
Experimental difficulty of the thesis, independence in experimental work	0-3	1
Quality of experimental data presentation	0-3	0,5
The use of up-to-date techniques	0-3	2
Contribution of the thesis to the knowledge in the field and possibility to publish the results (after eventual supplementary experiments)	0-3	2
Practical requirements – points in total		11
POINTS IN TOTAL (MAX/AWARDED)	48	(24)[2]

Comments of the reviewer on the student and the thesis:

The bachelor thesis of Stina Eßmann, deals with the subcellular localization and possible function of four chosen trypanosoma proteins. The thesis has 43 pages and consists of six parts (Introduction, aim of the work, Materials and methods, Results, Discussion and Conclusion), which are supported by appendix, list of abbreviation, list of literature and list of figures. This list (list of figures) seems useless for me, because it only contains figure legends. I do not see any reason why the reader shall pay attention to it because it will not bring any additional information to the legends present below each figure. The appendix contains information about used primers, buffer composition, antibodies, however this part miss some important information (will specify in my questions). I would also appreciate better structure of this part (eg. Appendix I, II) as it is a bit complicated to search for the information. The thesis contains various typos and incorrect sentences as well as improper statements (eg. Plasmids inoculation or Gibson transformation). I would suggest better proof reading prior submission next time.

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

Introduction: This part of the thesis is rather short (seven pages) and I would appreciate more pictures (e.g. schematic picture o Trypanosomatid cell would help the reader understand its complexity). I am also missing more information obout TAC (Tripartite attachment complex), which is crucial for the rationale of presented study, however the information here are limited.

Q1: How many proteins have been associated with TAC? You do speak about four in your introduction; however, there are more of them know from the literature. Is there anything known about the role of these proteins?

Material and methods: This part shows the methods author used during her work, however it is not written very well and many details important for individual methods are missing (eg. Primer concentrations, composition of digestive reactions etc.). This part also includes rather generic description of individual approaches, which is not necessary here and shall be omitted.

Q1: Can you explain me, what is stuffer? (I though this is a PCR amplicon, but in chapter 3.2.1 Preparation of RNAi plasmids you say that: "purified products were than mixed with the stuffer"

Q2: Can you specify what does individual steps in cell lysis program (table 2) mean?

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[2] Enter the number of points awarded.

Q3: Why did you linearize plasmids upon colony PCR?

Q4: What secondary antibody did you use for WB? What is the difference between antibodies used for IFA and WB?

Results: this part has eight pages and contains eight figures and one graph. I would appreciate better description of individual pictures, as it is often not clear (e.g. What are first two lines in figure nine or the remaining lines in figure 11).

Q1: Could author specify, which experiments were done by her and which were done by her supervisor?

Q2: you say that PCR for endogenous tagging was repeated 4times for gene Tb927.8.6970. I am wondering if you made any changes among the runs as the amplicon signal seems to be quite strong in figure eight.

Q3: You were getting quite low number of colonies upon Gibson assembly. Did you use any control to verify whether this is due to low level of assembled plasmids or improper transformation?

Q4: Can you show the SDS PAGE gel and whole WB membrane corresponding to figure 13? This way of presentation seems improper for me. What did you use for the SMOX control?

Q5: Can you please clarify, how many cell lines did you create during your work? From the thesis I am not sure if you create one V5 tagged line and another one for RNAi of you did RNAi for V5 tagged lines.

Q6: Can you show better picture of figure 14. There is almost nothing visible (e.g. phase contrast shows no cells). How would you comment on fact that WB signal for 5020 is very weak in comparison with other proteins, but IFA for 5020 is the strongest?

Q7: What are the other bands seen on WB for 5020 in figure 15?

Q8: What concentration of tetracycline did you use for RNAi experiments? Have you perform the kill curve prior this experiment?

Discussion: The discussion is written on 1,5 pages and the author discuss the differences in localization of individual proteins based on acquired results.

Q1: Author says that the cytosolic localization of 1440 and 1570 proteins may be due to low glucose conditions in growth media as shown for some other proteins by Bauer and Morris 2017. What was the glucose level of your media in comparison to the work of Bauer and Morris? Would there be any other possibility for presence of these proteins in the cytosolic fraction?

Appendix: Table presenting primer sequences shall be updated. It is not clear which primer is forward and reverse, It will be also nice to include gene specific parts for individual primers and which part of the gene is being amplified by PC. I am missing the information about the molarities of used primers. Would be good to specify antibodies you used (producer, cat. Numbers) as this can be critical for further experiments.

Q1: You do specify the composition of Gibson assembly MM. Did you make it by yourself? If yes, did you test its activity prior performing experiments (associated to Q3: results). Can you specify how much of individual components you add to one reaction (it is unclear from the table).

Q2: Composition of SDS PAGE gels is lacking important information about individual components (e.g. acrylamide-bisacrylamide ratio, SDS and APS concentration), what percentage gels did you prepare? All these information are important for proper gel casting. In addition, the preparation of SDS PAGE gel based on author´s description would result in suboptimal polymerization and is misleading to unexperienced reader.

Conclusion: The bachelor thesis of Stina Eßmann is rather weak despite the interesting topic. Upon reading the thesis, I did not get the feeling that the author understood all the methods she did use and I am convinced that the thesis could be presented in better way even when the author did not get many results.

In conclusion, I

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the thesis for the defense but will decide on the grade based on authors replies to my questions and on her performance during the defense.

In **České budějovice** date **10.09.2021**