



Přírodovědecká
fakulta
Faculty
of Science

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

OPPONENT'S REVIEW ON DIPLOMA THESIS

Name of the student: Bc. Eliška Kotounová, BSc.

Thesis title: Design and production of TBEV specific cleavage reporter for in vitro and in cell studies

Supervisor: RNDr. Zdeněk Franta, Ph.D.

Co-supervisor: Mgr. Tomáš Fessler, Ph.D.

Referee: RNDr. Martin Selinger, Ph.D.

Referee's affiliation: Department of Chemistry, Faculty of Science, University of South Bohemia

	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	2.5
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	2.5
Formal requirements – points in total		20

(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

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

Logic in the course of the experimental work	0-3	3
Completeness of the description of the used techniques	0-3	2.5
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	2.5
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		26
POINTS IN TOTAL (MAX/AWARDED)		48
		46

Comments of the reviewer on the student and the thesis:

The diploma thesis of Eliška Kotounová aimed to design and prepare reporter constructs for analysis of TBEV protease complex NS2B3. In more detail, two fluorescent proteins (PAmCherry and hMGFP) were fused with NS2B3 cleavage sequence and influenza M2 quencher protein. The resulting reporter cassette was either cloned to the pET19b/21b plasmids (protein production in *E. coli*) or pDEST40 plasmid (in cell studies in mammalian cell cultures). Eliška first optimized and then performed the production and purification of PAmCherry-M2 protein, which was subsequently used for in vitro analyses. In case of the “in cell” system, stable cell lines BHK-21 and Vero E6 producing either hMGFP-M2 or PAmCherry-M2 were obtained and subsequently used for the assays using TBEV strain Hypr.

Overall, the thesis is very well written and I greatly appreciate its coherence and an excellent graphic layout. The length of 66 pages (including the list of references and supplementary data) is more than satisfactory. The thesis is standardly divided into eight chapters: Introduction, Aims, Materials and Methods, Results, Discussion, Conclusions, References and Supplements. Honestly, I did not find any major mistakes, wrong formulations or missing information. Therefore, I have only few comments:

- 1) The linearization of pDEST40 plasmid before the stable cell line generation is not mentioned in Material and Methods chapter nor in Results. I would also check the presence of inserted DNA by PCR or the production of mRNA by qPCR or the protein itself by WB.
- 2) I would unify the composition of buffers in Table 2 (either mass (g) and volume (ml) or molarity); in my personal view the molarity is always better
- 3) I suppose there should be N-linked His-tag to hMGFP in pET19b described as construct 4 in Figure 9.
- 4) Next time, please ensure that the marker and sample will be on one image and not separated as in Figure 16.
- 5) I am also not convinced by the method used for antibiotic kill curve. This is hardly even semi-quantitative and there are plenty of quick and easy-to-perform viability-based methods. Moreover, the images of cells are very small and hard to “read”.

Questions, to which the student has to answer during the defense:

- 1) Are there any suitable alternatives to hMGFP/M2-quencher or PAmCherry/M2-quencher reporter systems in terms of other fluorescent proteins and quenchers?
- 2) Do you think that implementing an ER signal sequence into your cleavage reporter construct may help to increase the efficiency of the whole system?
- 3) How conserved is the NS2B3 cleavage motif among flaviviruses?
- 4) Was there any statistic evaluation of data in Figure 28 and 38? If yes, please describe. If not, please comment (I would assume that the step of calculating the signal-to-background ratio per cell could be considered as data normalization, right?).
- 5) Please, suggest any other method suitable for the detection of NS2B3-mediated reporter cleavage (for both *in vitro* and in cell studies).

Conclusion:

The diploma thesis of Eliška Kotounová is an example of excellent work summarized in a very well written and graphic form. Additionally, author proved an excellent knowledge of her field of interest by high quality discussion. Therefore, I **recommend** the thesis for the defense and I suggest the grade **excellent**.

In Umeå date 23.1. 2022



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signature



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OPPONENT'S REVIEW ON BACHELOR/DIPLOMA* THESIS

Name of the student: Eliška Kotounová, B.Sc.

Thesis title: Design and production of TBEV specific cleavage reporter for in vitro and in cell studies

Supervisor: RNDr. Zdeněk Franta, Ph.D.

Co-supervisor: Mgr. Tomáš Fessler, Ph.D.

Referee: Radek Šíma, Ph.D.

Referee's affiliation: Institute of Parasitology, Biology Centre, CAS, České Budějovice

	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	3
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	3
Formal requirements – points in total		21
(2) PRACTICAL REQUIREMENTS		
Clarity and fulfillment of the aims	0-3	3

* Choose one

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

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)		48
		(0-48) ²

Comments of the reviewer on the student and the thesis:

In this thesis, Eliška Kotounová developed a genetically encoded dark-to-bright fluorescent reporter which is activatable by the TBEV NS2B-NS3 protease, and tested its utility for virus replication in living cells. Formally, the thesis follows the common structure required by the Faculty of Science. It is composed in a logical manner and subdivided into sensible sections. Eliška had to master a broad spectrum of methods which is a strong part of her master thesis and important input for her future career. Eliška Kotounová prepared a high-quality master thesis. It contains a minimum of mistakes, and I appreciate that she decided to write her thesis in English. Eliška proved her talent for scientific work, and I am sure that she will employ gained experience in her future research career. I'm happy to **recommend** her master thesis for defense.

Suggestions and questions, to which the student has to answer during the defense.

Mistakes, which the students should avoid in the future:

Q1. In Table 16, I was surprised by the high annealing temperatures of the primers amplifying hMGFP (70 °C) and pUC19_inhib (75 °C). This is quite unusual for 18 (24) bp primers. Could you please comment on that?

Q2. In chapter "4.2.2 Antibiotic kill curve", the author tested the sensitivity of chosen cell lines to geneticin (G418) to find optimal concentration for the establishment of stable cell lines expressing the reporters. The optimal concentration of G418 was taken as the lowest concentration that killed all the cells within 14 days. Based on these results, the concentration of 0.4 mg/mL was determined as optimal and was used in further experiments. But Figure 25 shows that there are still living cells in the culture at a concentration of 0.4 mg/mL. All cells are dead at a concentration of 0.6 mg/mL. So why did you choose a concentration of 0.4 mg/mL and not 0.6 mg/mL?

Conclusion:

In conclusion, I **recommend** the thesis for the defense and I suggest the grade **excellent (1)**.

In České Budějovice, January 22nd, 2022


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signature

