Přírodovědecká fakulta
Faculty of Science

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

STATEMENT OF THE BACHELOR THESIS OPONENT

Name of the student: Simona Fiserova
Study program: **Biological chemistry**
Department/Institute: **Deaprtment of molecular biology, University of South Bohemia**
Thesis title: **Interaction of protein Clock and Cycle - Preparation of constructs for subsequent expression in S2 cells**

Supervisor: **David Doležel, Ph.D.**
Supervisor`s affiliation: **Biology Centre, Institute of Entomology, Czech Academy of Sciences**

	Point scale[1]	Points
(1) FORMAL REQUIREMENTS		
Formal and graphical quality of the thesis	0-3	3
Ability to work with literature	0-3	3
Language and stylistics	0-3	3
Formal requirements – points in total		9
(2) PRACTICAL REQUIREMENTS		
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 results and their discussion with the literature	0-3	2
Experimental difficulty of the thesis, independence in experimental work	0-3	2
Contribution of the thesis to the knowledge in the field and the possibility to publish the results (after eventual supplementary experiments)	0-3	2
Practical requirements – points in total		12
POINTS IN TOTAL (MAX/AWARDED)	21	(0-24)[2]

[1] Mark as: 0-unsatisfactory, 1-satisfactory, 2-average, 3-excellent.
[2] Enter the number of points awarded.

Comments of the oponent on the thesis:

Simona Fiserova thesis aimed to verify plasmids containing Clk and Cyc genes and then perform a reaction using the Gateway system in order to create expression plasmids containing the Clk or Cyc genes under two types of promoters and two types of tags. These vectors were then ready to use in S2 cells to study the physical interactions of these proteins during the circadian rhytms.

Simona's thesis is nicely written, with clear structure, well composed introduction, result section and discussion. It is obvious that she understood the purpose of the project as well as details of the experiental setup. She learned to work with bacteria, isolate plasmids, perform the Gateway cloning and verify the resulting plasmids by sequencing reaction, comparing the results to the in silico cloning in the Geneious program; all these methods will be useful to her in any future molecular biology project. She certainly devoted enough time for writing up the thesis, therefore there is hardly anything left for the reviewer to criticise. I only found a few tiny bugs that I mention; these are minor and do not spoil at all the overall very good impression of the thesis.

- p 16: missing information in the picture about the resistance of the Entry clone vectors. Explain how do you distinguish the expression clone from the destination vector and entry vector present also in the ligation mixture.
- p19: missing information in the Result section: which primers were used for checking the sequence of the att sites in the destination and entry vectors?
- p 20: the pAGW, pHGW and pARW vectors could have been mentioned in more detail in the Method section, before showing the result of the in silico cloning in the Results
- p22: missing information which primers were used to check bacterial colonies by PCR and what was the expected theoretical size of the product

Specific questions that Simona can address during her defence, based on her knowledge and information from the literature:

- You decided to clone the genes with an N-terminally fused mCherry/GFP tags. Could a tag placed on N terminus of the protein interphere with its proper localization / function / binding partner selection?
- Does the stability of the Clk or Cyc proteins or their mRNAs play a role in circadian rhytms? Could the tag make the protein more stable than wild type, interphering with its proper function? Alternatively, do the UTR regions of the endogenous mRNA for these proteins regulate the stability of mRNA that would be important for the circadian rhytmicity? If yes, should this be taken into account when cloning into expression vectors?
- Do you think that the level of expression of the Clk or Cyc gene does matter for the circadian rhytms? How much are the genes normally expressed in S2 cells? Could too strong overexpression interphere f.e. with the Per/Tim binding?

As I wrote before the thesis is of excellent quality and Simona certainly achieved the aims she set. My only concern is that the aims could have been a bit more ambitions to my opinion. The experimental part was simple and it was probably achieved in only few weeks. I would have appreciated if Simona proceeded a bit further, for example if she tested the functionality of the new vectors in S2 cells. At the same time, I understand that the time allocated to the Linz student is rather short and considering the amount of exams they need to take at the same time there is not much time left for the actual bench work. Given this consideration the amount of work Simona did is sufficient for the bachelor thesis although it is less than one would expect from a regular bachelor.

Conclusion:

In conclusion, I r e c o m m e n d the thesis to be defended with the grade excellent.

In **Ceske Budejovice** date **29th May 2017**

.....

signature