Přírodovědecká fakulta
Faculty of Science

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

STATEMENT OF THE BACHELOR THESIS REVIEWER

Name of the student: **Daniel Kitzberger**
Thesis title: **Optimization of CRISPR/Cas9 technique for the *Ixodes* ticks genome editing**
Supervisor: **RNDr. Jan Štěrba, Ph.D.**
Co-Supervisor: **Mgr. Kateryna Kostarenko, Ph.D.**
Reviewer: **Mgr. Jaroslava Lieskovská, Ph.D.**
Reviewer`s affiliation: **Department of Medical Biology, Faculty of Science, University of South Bohemia, 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	2.5
Formal requirements – points in total		20.5
(2) PRACTICAL REQUIREMENTS		
Clarity and fulfillment of the aims	0-3	2
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	1
Logic in the course of the experimental work	0-3	3

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

Completeness of the description of the used techniques	0-3	2
Experimental difficulty of the thesis, independence in experimental work	0-3	2
Quality of experimental data presentation	0-3	2
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	1,5
Practical requirements – points in total		19.5
POINTS IN TOTAL (MAX/AWARDED)	**46**	**40**

Comments of the reviewer on the student and the thesis:

Thesis written by Daniel Kitzberger deals with the optimization CRISPR/Cas9 technique for *Ixodes* ticks genome editing. This genetic engineering technique, based on simplified version of the bacterial CRISPR/Cas9 antiviral defense system, is relatively new method used lately quite often for knock-out or knock-in of chosen genes. The usage of method has a few restrictions, for example the knowledge of genomic and transcriptomic sequence is required and that is unfortunately not fully known for neither *I.ricinus* nor *I.scapularis*. The task was thus challenging. The aim of the thesis was to find proper target sequence within genes of interests, and then to prepare ribonucleoprotein complex (consisting of so called single guide RNA and Cas9 endonuclease) to edit target DNA. The author had to performed the bio-informatic analysis of genomic and transcriptomic sequences and several common method of molecular biology.

The thesis is well written. The literature overview is the best part of the thesis and consists of a clear description of principle of this method and also includes a research that led to the development of this technique. Thesis is supplied with several figures that very well document the text information about studied topic and methodological approach. The aims are fine but it would be clearer if instead of genome editing and target genes author used more specific words.
As concerns the results, the experimental work probably did not come up to full expectation since produced ribonucleoprotein complex (RNP) did not cleaved the target alpha 2,6-sialyltransferase gene. Still I think it had to be exciting to learn this technique and more details about it. Discussion is the weakest part of the thesis. It should contain a comparison of obtained data with literature or the attempt to explain why it did not work with reference to other article. No reference was unfortunately present in the discussion.

In spite of it I think the thesis has overall a good quality and work done by Daniel Kitzberger was beneficial for research project.

Comments:

1. Method describing the preparation of denaturing urea polyacrylamid gel is missing.
2. In description of methods there is combination of present and past tense. Should be unified.
3. Figure 5 would require better labeling.
4. It is proper to explain the meaning of abbreviations before first use.
5. Limit the usage of abbreviations to minimum.
6. I never experienced before that expected results were replaced with made image. I

understand that the purpose was to demonstrate what we should see so I accept it. But it is possible that not all reviewers would have same opinion. So I recommend avoiding it in the future.

Questions:

1. What is the potential function of DAMT, DNMT1 a DNMT3?
2. In Figure 5 there was no PCR product obtained from *I. scapularis* genomic DNA while PCR product of correct size was seen from ISE cell line. Can you comment it please?
3. On page 28 and 29, there is presented by two methods that prepared single guide RNA does not have right size, instead looks like smear of smaller RNA. I think it was not correct to expect that such sgRNA would not affect efficiency of ribonucleoprotein (RNP) complex formation as is stated in text. Do you agree with me?
4. What was your involvement in bioinformatic analysis? Have you done it independently?
5. CRISPR/Cas9 technique has been used in human medicine. Can you give us some examples?

Conclusion:

In conclusion, I **r e c o m m e n d** the thesis for the defense and I suggest the grade 2.

In České Budějovice date 20. 1. 2021.