Přírodovědecká fakulta
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Review of the Master thesis submitted by Bc. Dominika Reichensdörferová

Development od DiCre parental lineage of *Babesia divergens*.

Supervisor: RNDr. Daniel Sojka Ph.D.

Reviewer: RNDr. Zdeněk Franta Ph.D. Faculty of Sciences, University of South Bohemia, České Budějovice

The Master thesis of Bc. Dominika Dominika Reichensdörferová deals with very interesting topic of conditional gene knock-out of piroplasmid parasite *Babesia divergens*. The authors main task was to introduce the new technique into the lab and establish it *de novo* for a babesia sp. parasite. Such goal is in my eyes quite challenging and could be easily a part of a PhD work. The author had to optimize/adapt many key approaches for herself (e.g. selection antibiotics kill curve, DiCre cassette design), as there is no study published so far. The thesis is written on 50 pages and consists of eight parts (introduction, objectives, material and methods, results, discussion, conclusion, references and appendix). The thesis reads well and does not contain many typos. I appreciate that the author decided for writing the thesis in English.

The introduction is written on 11 pages and guides the reader from the general information about the Babesia genus through the disease caused by the parasite towards the functional genomic and DiCre system. The chapter contains three figures, which have a good quality and help the reader to orientate in and understand the text.
I have two questions / comments to the applicant:

1 – On page three you state that "The tick has a three-host life cycle, spanning three years…". This statement a bit misleading, cause hard tick can finish its life cycle within a year.

2 – Is there anything known what triggers the sporoblast maturation upon tick attachment?

Material and methods chapter has eight pages and contain all the techniques that candidate used during her research. Because it is a master thesis, this part shall be written in more detail. Some methods (e. g., chapter 5 DNA cloning and sequencing) are not described in sufficient detail and would not allow the reader to repeat the experiment.
I have two questions to this part.

1 – Is there any reason for isolating the DNA from the culture of 5-10% parasitemia? You can easily reach 20-30% and I would expect you get more DNA out of it.

2 – Can you specify, what do you mean by biological triplicate? According to your description in chapter 3 of MM it seems to me more like technical triplicate.

The results are written on twelve pages and are supported by nine figures. This chapter follows the goals of the diploma thesis. The first part of results, dealing with the cell culture experiments, shows the effect of tested compound on parasite growth and is well supported by experiments, However, the second part of results (starts with chapter 4 DiCre cassette vector design) is rather theoretical. It does not contain a single PCR gel nor any proof that the author really perform the experiments. All the figures here are just schemes without any evidence for the experimental work. Could the author include experimental figures for here defense and guide us through the whole experimental design and acquired outcomes?
My questions / comments to this part:

1 – In figure 6, you show the effect of WR99210 on *Babesia divergens* parasite. How does the drug work? What is its mode of action? From this figure, it seems to me, that the effect of the drug starts to be visible at day 8 (you see dose dependency in measure parasitemia). Have you tried to monitor the culture for longer period?

2 – In figure 8, you calculate the IC_{50} for WR99210 to be ca 2,5nM. I am missing this concentration in figure 6 (you used 5nM as well as 1,25nM). Did you test the drug around the IC_{50}? I would be also careful when plotting the curve, because you go to negative parasitemia, which is incorrect!

3 – In figure 9, you show the effect of G-418 on parasite growth. Do you have any explanation for the drop of parasitemia from day 4 to day 6 followed by its increase? It is also interesting that three G-418 concentrations (205, 500 and 750 ug/ml) show higher parasitemia than negative control. Have you tried to repeat the experiment? If not, I would recommend doing the kill curve again, because its results would strongly influence calculation of IC_{50} for G-418 (figure 11).

4 – The rationale for choosing Bdiv_04560c segment as a recipient locus is mentioned in the discussion chapter only. The bioinformatics analysis behind the identification of this locus could be part of results, as it is one of the main goals of the thesis. Did you test for any other suitable locus?

5 – Did you design the DiCre cassette by yourself? Why did you chose the individual parts (e. g., promotors)? Was it based on already published work? I would also suggest specifying the individual restriction sites in the scheme. It will be more informative.

6 – You say that the DiCre cassette vector was ordered as a synthetic one, but the company did not succeed. From this reason you tried to assemble it by yourself, but from presented data it is also not clear, whether you managed or not (it is all schematic!). You state, that you did use two strategies (1 fragment vs 2 fragments) for Gibson assembly. Which one did finally work? How did you verify the correct insertion of individual parts?

The discussion is on four pages and compares the acquired results with available literature and offer possible outlook in the use of DiCre system for babesia sp.

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Finally, as already stated, my biggest criticism goes to the Results part. I am really missing any experimental data, which has to be included. Without them, it is not clear whether author performed all the experiments and fulfilled the goals of her diploma thesis. Despite all my comments and criticism, **I recommend** the thesis for the defense but I will suggest the grade based on candidate's performance during presentation and answers to my questions

In České Budějovice, 18.05.2022 Zdeněk Franta

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