KATEDRA MOLEKULÁRNÍ BIOLOGIE A GENETIKY
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STATEMENT OF THE MASTER THESIS REVIEWER

Name of the student: Bc. Václav Brabec
Thesis title: *In silico* and functional analysis of clock component *timeless-m*
Supervisor: Mgr. David Doležel, Ph.D.

Reviewer: Mgr. Lenka Chodáková, Ph.D.

The study of Václav Brabec aims to understand the role of *tim-m* in the circadian clock of *Drosophila melanogaster*. By using the tools of CRISPR/Cas9 mutagenesis, he was able to create a series of viable homozygous mutants with alterations in several features of the clock.

At the beginning I would like to state, that as this is the first master thesis I had a chance to review, I wish in the future all theses would be like this. I enjoyed the reading and I was impressed by the amount of performed work. I have to give the author some feedback, but the following is just minor critique.

The thesis is written in a good English, sometimes with a bit quirky structure, which only gives me an impression, that the thesis was written independently with the use of the authors own words. I will not focus on several mistakes in the text as they were sent to the author, so it can be (maybe) used during the manuscript preparation.

The **Introduction** gives us an idea about the problematics; however, I have a problem with the choice of the style of the written text, as I would more appreciate to read the introduction as a "story" rather than a "list" of little disconnected topics.

Notes: - page 5, second paragraph: "As was mentioned in chapter 1.5…" should be "…chapter 1.3"
- page 5, second paragraph: "…whereas the mammalian clock is based on the cooperation between PER and CRY-m (Kotwica-Rolinska et al., 2022)." – that is not an original citation.

What I greatly value is the **Material and methods** section, which shows a large amount of learnt techniques and work performed.

Q: How long can you store squished DNA?
You write: "The entire sequence of exon 18 was pasted into the flyCRISPR,…" – Why exon 18?

On page 13 you write: "…three gRNA with the optimal characteristics were selected." – What are the optimal characteristics?
Can you explain why you screened the gonads? Could you have avoided this step?

Notes: - page 12, primer concentrations are missing – also on couple of places in the text there is "…(concentration is important)" in parentheses ☺
- page 13, Table 3 says: "gRNA sequence" – which should rather be "gRNA-coding DNA"
- page 14: missing primer concentrations.
- page 14: you don´t have to write the kit instructions in the text.
- page 21: in the text you mention figure 1.1 – it should be figure 7.
- page 22, 3.9. Mosaic fly screening. – you write: "It used the same PCR program as in tim2 PCR verification." – what does that mean?
- ActoJ should be cited
- I think the PCR mastermix (Top Bio) is 2x concentrated.

The **Results** section is well structured, with results and figures described in detail. I have a big problem with not referring to figures in the text, which often confused me (figures 13-17 are not referenced at all). I understand what you were trying to do, that you write the text and then put a figure underneath it - but you shouldn't do that. Also, I am kind of familiar with the principle of heteroduplex PAGE screening, but maybe it would be nice to put it in a figure so it would be easier to understand.
Also, figure 27 is not discussed in the text at all, it just appeared there. There is a good explanation what is happening in the figure underneath it, but it should be clearly explained, why did you perform the experiment (as it is also not mentioned in the M&M section).
What I was a little unhappy about was the naming of the mutants, I often got lost and had to go back through the text to find a specific strain and its characteristics.

Q: What was the genetic background of the flies injected with the plasmids?
Is the use of CANTON-S flies as WT control correct?
What are the features/phenotype of the tim-d^{ritsu} flies?
Did you manage to create some flies, which were homozygous lethal?

Notes: All of the figures with the electrophoretic gels have ladders with unknown size.

Discussion and conclusions are written well. Only minor thing is, that I would not call the protein alignment an "experiment which identified an evolution of a gene structure" or "the phylogenetic analysis". One last thing: I would appreciate if the author would not use statements as "The study results showed **some** involvement of *tim-m* in the fruit fly clock…". It gives a somewhat uncertain impression of the author with the result.

From what I have read in the thesis, I believe that the author did very well in the lab and learned many techniques, which is probably the most important goal of a master's degree.
In conclusion, I would like to say that the thesis is very good and I recommend it for defense.