Review of the bachelor thesis

"CRISPR/CAS9 Genome editing in *Pyrrhocoris apterus*"

by **Maly Bertolluti**

The present thesis deals with design of single-guide RNA targeting the C-terminal part of the *Cry2* gene in *Pyrrhocoris apterus* to introduce mutations in this region and eventually test for its function. The thesis is written in English, consists of 33 pages and is traditionally organized into Introduction, Aim, Material and methods, Results, Discussion, Conclusion, and Literature. The text is supplemented with 15 figures and 4 tables.

In Introduction, the author provides information on CRISPR/Cas9, model organism, and circadian clock. The Aim is clearly defined. Methods are described in sufficient details. I appreciate that the whole workflow is described although the student did not perform injections and analysis of F1 progeny of mutants due to long generation time of *P. apterus.* Results are well described and documented with figures and tables. However, in some cases text does not refer to relevant figures. Discussion compares predicted efficiency of sgRNAs to empirical ones. I would also welcome comparison of hatchability and survival rates between mutants (as listed in the Table 4) and wild types or controls. In Conclusion, the author comments on versatility and time-effectiveness of CRISPR/Cas9 for functional studies in non-model species. I have folowing comments and questions:

Introduction

- The Figure 2 shows mutations introduced after repair of a double strand break introduce by CRISPR/Cas9. The figure was reproduced from a web page which is correctly cited. However, is the depiction of deletion correct?
- "SpCas9 has a PAM sequence of 5'-NGG-3' where N can be either Adenine, Thymine or Guanine." (p. 4) Should not it be 5'-DGG-3'?
- Abbreviation "sgRNA" is used for both short- and single-guide RNA in the text. Are these terms equivalent?
- How is a dimer of Per and Cry transferred back to nucleus?
- References in figure legends are in different format.
- Figure 6 shows relationships within the cryptochrome gene family. What are DASH proteins at the basis of the tree? Did the *Cry* paralogs evolved multiple time independently or were they duplicated in a common ancestor and lost in some taxa?

Methods

- I think sequences of the *cry3* forward and reverse primers (p. 13) used for verification of mutants do not match those annotated in the Figure 11.
- Why was CRISPOR prediction performed with the "No Genome" option when genome sequence is available?
- In the CRISPR Forward primer, complementary part is followed by a nucleotide sequence missing in designed sgRNAs. I assume this is an error. What is a function of 5'-GAAATT before the T7 promoter?
- Composition of in vitro transcription reaction is written down in volumes without stating concentrations and thus not reproducible.

- It is not clear to me how is heteroduplex formed in a heteroduplex formation assay. I believe samples need to be denatured before gel separation.

Results

- In some cases text does not refer to relevant figures (4.2 does not refer to Figure 8, 4.3 to Figure 9, it is not referred to Figures 12, 13, 14)
- Nucleotide and protein sequence are not readable in Figure 10.
- What does the author mean by "Predicted double stranded breaks made by the Forwrd primer is indicated by the dark green box... (p. 22)"?

Discussion

- Figure 13 in Discussion should be labelled as Figure 15.

Literature

- web pages should be listed separately
- Latin species names should be in italics
- Jamal et al. 2016 is missing DOI and issue number

Conclusion

Despite minor issues listed above, I really liked the thesis, which provides nice and detailed primer into CRISPR/Cas9 mutagenesis. The author acquired experience with state-of-the-art methodology and obtained original results which were included in a recent publication. The text is written in good English, although there are some typos and stylistic errors common in bachelor studies. Overall I am impressed by the results obtained considering that students of the Biological chemistry program do not have so much time to work on their theses. The present thesis thus in my opinion fulfils all requirements and I recommend it for successful defence.

On January 27, 2020, in České Budějovice

Petr Nguyen