Andrew Samuelson, Ph.D. Research Associate Professor



March 13, 2018

Vineeta Bajaj, Ph.D. Review Editor JoVE 617.674.1888

RE: manuscript, JoVE57819

Dear Dr. Bajaj, Ph.D.

Thank you for your continued consideration of our manuscript entitled: "The Replica Set method: a high-throughput approach to quantitatively measure *Caenorhabditis elegans* lifespan" for publication in JoVE. We thank the editors for their time in facilitating the conversion of our manuscript to a format suitable for JoVE. We appreciate their guidance, and in the revised manuscript we have attempted to address all of the critiques. We hope the manuscript is now suitable for acceptance in JoVE.

In the revised manuscript we have added a paragraph to the introduction that explains in some detail how feeding based RNAi is achieved in *C. elegans*. There was some confusion by the editor as to how RNAi works, which is reasonable for anyone unfamiliar with *C. elegans* functional genomics. Additionally we have expanded the protocol on hypochlorite treatment for synchronizing animals at the start of a lifespan experiment and moved to discussion other options for synchronization. Similarly we have stream-lined the protocol portion for the use of FUdR as a method to prevent progeny production and expanded our discussion accordingly. We provide additional point by point responses in the detailed response to the critique below.

Thank you again for your time and consideration of our manuscript. We look forward to hearing from you.

Sincerely,

Andrew V. Samuelson

Detailed response to the critique.

1. The editor has formatted the manuscript as per journal's style. Please retain the same.

We have maintained the formatting during revising.

2. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., "Do this," "Ensure that," etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as "could be," "should be," and "would be" throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a "Note." However, notes should be concise and used sparingly. Please include all safety procedures and use of hoods, etc.

We have made additional revisions to address this, with particular attention to passages that were alluded to by the editor in comments.

3. The Protocol should contain only action items that direct the reader to do something. Please move the discussion about the protocol to the Discussion.

We have made these changes as suggested in the new revision. Specifically, in the revised manuscript protocol section we now outline chronological synchronization through the use of hypochlorite treatment and preventing progeny through the addition of FUdR. We have expanded our discussion to include other ways to synchronize animals, and other methods to handle the issue of progeny.

4. The Protocol should be made up almost entirely of discrete steps without large paragraphs of text between sections. Please also ensure that there is enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol.

Longer passage have been trimmed, or broken down into further steps where possible.

5. Please remember that the protocol cannot be more than 10 pages in length with all the formatting and headings and spacings.

We were not previously aware that the page length limit included whitespace. We have heavily edited the protocol section to restrict the page length to 10 pages.

6. Please also check the highlights to not be more than 2.75 pages in length.

Similarly, our estimate of the length was not including whitespace previously, so we have changed the highlighted selections accordingly. In the revised manuscript only two pages of text are highlighted.

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Manager account. The Figure must be cited appropriately in the Figure Legend, i.e. "This figure has been modified from [citation]."

We have looked into copyright permission for the relevant figures. Neither figure that has previously been published requires explicit permission for reuse.

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Note: Figures 1-3, and S1 are new.

8. Please upload the Tables as supplementary files individually.

We have made this change to the arrangement of the tables and renamed them as Supplementary Files.

9. Please replace the word "wormlife" with a more generic term as much as possible throughout the entire manuscript. Specific product names can be introduced or used infrequently if directly relevant but should not be used to the point where the protocol reads like an advertisement. You can certainly introduce the software but we ask the authors to reduce the instances to remove any bias.

The number of mentions of the name of the software have been substantially reduced. We have only retained the word wormlife in a few instances (when referring to the software as a whole). Other instances where it has been retained are limited to cases where we are directly referring to a file name that is necessary to use the software.

10. Please address specific comments marked in the manuscript as well.

We have made modifications as appropriate in response to the comments in the manuscript and have replied to some comments with the review tools.