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Dear JoVE,

We are looking forward for this opportunity to have our manuscript published in your journal. We believe that the comments provided by the editorial staff and also the reviewers have greatly strengthened the manuscript. We thank both the editors and reviewers for their comments. Below we have provided the original comments that were provided to us and below each comment/suggestion we have placed our response in red text. We look forward to hearing back from you and hopefully moving to the next step of this manuscript.

Please don't hesitate to contact me for any additional information.

Thank you,

Afshin Beheshti, PhD

Editorial comments:
Changes to be made by the Author(s):
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

We have gone through the manuscript and thoroughly made edits from our proofreading.

2. Please obtain explicit copyright permission to reuse any figures from a previous publication. Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints. Please upload this information as a .doc or .docx file to your Editorial Manager account. The Figure must be cited appropriately in the Figure Legend, i.e. "This figure has been modified from [citation]."

Figures 1 and 6 were modified from figures originally from our publication: Beheshti et al, Scientific Reports, 2018 (reference # 35 in the manuscript). According the Scientific Reports and their licensing agreement and copyrights, users are free to share modify the figures in the publication. Here are the links of their editorial policy addressing this:

https://www.nature.com/srep/journal-policies/editorial-policies#license-agreement
https://creativecommons.org/licenses/by/4.0/legalcode

3. Figure 3: Please provide a figure with higher resolution if possible.

We have provided a higher resolution image for figure 3.

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4. Please provide an email address for each author.

We have provided email addresses for all authors.

5. Abstract: Please do not include references here.

We have removed the reference from the abstract.

6. Please rephrase the Introduction to include a clear statement of the overall goal of this method.

We have added sentence at the beginning of the introduction to provide a clear goal of the methods for this manuscript. The following was added:

"The overall goal of this manuscript is to provide a clear methodology of how to use NASA's GeneLab platform and how rodent experiments done in space is translated to data to be analyzed in GeneLab."

In addition, the last paragraph of the introduction also discusses the goal of the manuscript.

7. Please revise the protocol text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).

We have gone through the protocols text and removed all personal pronouns.

8. Please adjust the numbering of the Protocol to follow the JoVE Instructions for Authors. For example, 1 should be followed by 1.1 and then 1.1.1 and 1.1.2 if necessary. Please refrain from using bullets, dashes, or indentations.

We adjusted the numbering of the protocol to follow JoVE instructions. We also removed all indentations.

9. Lines 97-113: The Protocol should be made up almost entirely of discrete steps without large paragraphs of text between sections. Please simplify the Protocol so that individual steps contain only 2-3 actions per step and a maximum of 4 sentences per step.

We have modified this section to be discrete steps.

10. Please revise the protocol to contain only action items that direct the reader to do something. 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"

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throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a "Note."

We have revised the protocol section so no imperative tense is present.

11. For computational steps, please provide software screenshots as supplementary files to match each step.

We have provided additional supplemental figures from throughout the computation steps.

12. Please include single-line spaces between all paragraphs, headings, steps, etc.

We have provided single-line spaces between all paragraphs, headings, and steps.

13. There is a 2.75 page limit for filmable content. Please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.

We have highlighted in yellow the 2.75 pages that should be filmed.

14. Please ensure that the highlighted steps form a cohesive narrative with a logical flow from one highlighted step to the next. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense.

The highlighted steps match the guidelines listed above.

15. As we are a methods journal, please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations:

a) Critical steps within the protocol
b) Any modifications and troubleshooting of the technique
c) Any limitations of the technique
d) The significance with respect to existing methods
e) Any future applications of the technique

We have edited the discussion and added 2 more paragraphs that include the critical steps of our protocol, how potential modifications can be done to the any of the steps, any limitations the technique, significance, and future applications. We believe that these additions to the discussion have provided for an overall stronger manuscript.

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16. References: Please do not abbreviate journal titles.

We revised the references so the journal titles will not be abbreviated.

Reviewers' comments:

Reviewer #1:
Manuscript Summary:
The NASA GeneLab platform provides access to omics data from biological spaceflight experiments. The authors described how a typical mouse experiment is conducted in space and how data from such experiments can be accessed and analyzed using GeneLab.

Major Concerns:
none

Minor Concerns:
The manuscript, although complete and very clear, presents the need for a minor revision oriented as suggested below:
1. There are several typos: it is suggested to reread the whole manuscript carefully to correct them.

We thank the reviewer for this comment and have gone through the manuscript thoroughly to correct for the typos.

2. Rows 52-55: please include also refs to bone studies (example but not exclusive: Giuliani A, S, Ruggiu A, Canciani B, Cancedda R, Tavella S. (2018) High-Resolution X-Ray Tomography: A 3D Exploration Into the Skeletal Architecture in Mouse Models Submitted to Microgravity Constraints. Frontiers in Physiology. 2018;9:181. doi:10.3389/fphys.2018.00181.)

We have added the reference the reviewer has suggested.

3. Rows 55-58: Please include and discuss also on the MIS habitat. Ref. Blottner D, Serradj N, Salanova M, et al. (2009) Morphological, physiological and behavioural evaluation of a 'Mice in Space' housing system. J Comp Physiol B. 2009 May;179(4):519-33. doi: 10.1007/s00360-008-0330-4.

We have added the reference the reviewer has suggested.

4. Rows 290-291: "Upload the list of genes with fold-change values for the statistically significant genes determined in step 3.3.". Please verify if you really referred to step 3.3.

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We thank the reviewer for catching this mistake. We have updated this step to refer to step 9.4.4.

Reviewer #2:

Manuscript Summary:

The manuscript gives an example of how to use Genelab to analyze data from spaceflight. The strength of the article is the thorough explanation on how to use Genelab. The use of the specific example of vivarium versus rodent habitats makes this explanation of Genelab clear and interesting.

Minor Concerns:

The article aims to accomplish two goals: spread the word on how to do spaceflights experiments using rodents, and describe to new users how to use Genelab to make new discoveries. The article works well for these two objectives for the initiated user, i.e. scientists who have a passing or intimate familiarity with doing science in space. However for scientists new to space science, I recommend that the authors add text to circa line 108 , and possibly to the section 'Representative Results' to expand on the crucial importance of ground controls for space research, and the importance of continuously mirroring as many environmental factors as possible in the ground controls.

We thank the reviewer for the above the comment. This is a very good point and we have added the following text in the sections the reviewer suggested:

This text was inserted after what was formerly line 108 (currently line 134):
"1.3.1 Note: It is important to note that ground controls are essential for spaceflight rodent experiments. As described in this protocol, these controls are done with both identical conditions (i.e. CO2 conditions, humidity, temperature, cage dimensions, etc.) in the Rodent Habitats and also in normal vivarium cages that have standard environmental conditions on Earth. The rodents housed in the Rodent Habitat ground controls allow for direct comparison to rodents in space. While rodents housed in vivarium cages allow for biological comparison between the different environmental conditions between habitats."

We also added in the 'Representative Results' section the following as the reviewer has suggested (starting from line 502):

"For this study we primarily focused on the biological differences that occur in rodents housed in the Rodent Habits ground controls and the vivarium controls. As described above, it is key to have better understanding of these two habitats, which will provide us information on possible confounding factors that can impact health due to the environment on the ISS. For all rodent spaceflight experiments these ground controls are also essential to determine which biological factors are associated directly with spaceflight or due to the environmental conditions on the ISS."

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My second recommendation is then to add text to the section 'Representative results' that explains clearly that the data that was analyzed was from the two ground controls, i.e. Rodent Habitat control and the vivarium control. Also, I assume that the vivarium control was not exposed to elevated CO2. But were other conditions (e.g. temp and humidity) identical? This needs to be made crystal clear.

We have added the following starting on line 509 to address the above comment and make it clear that only the CO_2 is different between the Rodent Habitat and vivarium control:

"The vivarium habitat has the normal CO2 level that is present on Earth (currently being ~300 to 380ppm). The temperature and humidity for both habitats are similar."

There is a risk, because of the detail give to protocols for doing animal experiments in space that readers (viewers of the video) will assume that spaceflight data were analyzed in the example. Because of this potential for confusion, it might be worth considering changing the structure to the following order: 1) importance of doing research on spaceflight risks; 2) example of rodent research and importance of good ground controls; 3) example of vivarium data compared to rodent habitat data using genelab; 4)details on how to use Genelab to do this kind of analysis; 5) detailed protocol for doing rodent research in flight.

We have added the following at the end of the introduction (line 103) to clarify the data we present as an example for the analysis will be specifically related to differences between the Rodent Habitat and vivarium controls:

"The specific example we will present on how this protocol is implemented will be comparing the biological differences occurring in rodents housed in Rodent Habitat and the vivarium controls that was published by Beheshti et al.[35]"

We have also reorganized the 'Representative Results' as the reviewer has suggested. The text that we have added to address the above comments are incorporated and organized into this suggestion.