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Vineeta Bajaj, Ph.D.  
Review Editor  
Journal of Visualized Experiments

October 30th, 2018

Dr. Vineeta Bajaj,

We thank the editors and reviewers for their constructive comments on the manuscript and have made edits to address their points.

In particular, all commercial language has been removed from the manuscript and confined to the Table of Materials.

We believe the manuscript is now suitable for publication in JOVE.

Thank you.

Christine Cheng



Assistant Professor

Department of Biology

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***Editorial Comments***

***UPDATED 11/5/18***

*1. The editor has formatted the manuscript to match the journal's style. Please retain the same.*

We thank the editor for these corrections.

*2. Please address all the specific comments marked in the manuscript.*

These comments have been addressed in the manuscript or here in the rebuttal.

*3. Please change the title to reflect the highlighted portion of the protocol.*

The emphasis on low mitochondrial DNA contamination in the title is necessary. Modifications to the original ATAC-seq protocol that we demonstrate are all aimed towards this goal of reducing mitochondrial contamination, and will be the primary interest for readers. We have deleted a description of ATAC-seq from the title instead to make is shorter.

*4. Notes cannot be filmed hence highlighting is removed. Highlights are adjusted to form a cohesive story. Please check.*

The highlighted script forms a cohesive story.

*5. Please refer to the figures in the order of their numbering. So figure 1 should be referred before 2 and so on. Please do the same with the tables as well.*

Thank you for bringing this to our attention. The error has been fixed.

*6. Please include a title and a description of each figure and/or table. All figures and/or tables showing data must include measurement definitions, scale bars, and error bars (if applicable).*

A title has been clarified or each figure and table.

*1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.*

The manuscript has been carefully reviewed.

*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].”*

Copyright permission has been obtained and a link to the editorial policy to allow re-prints uploaded to the Editorial Manager account. Figure legends are cited appropriately.

*3. Figure 1: Please remove commercial language (Tween20).*

Commercial language has been removed.

*4. Figure 4: Please change the time unit “hr” to “h”.*

Time unit has been corrected.

*5. Please upload each Table individually to your Editorial Manager account as an .xls or .xlsx file.*

Apologies for the confusion on this point, tables have been uploaded as separate documents to the Editorial Manager.

*6. Tables: Please use the micro symbol µ instead of u and use the temperature unit (°C) instead of “C”.*

Micro symbols and temperature units have been corrected.

*7. Table of Equipment and Materials: Please sort the items in alphabetical order according to the Name of Material/ Equipment.*

Table of Materials has been sorted alphabetically.

*8. Please provide an email address for each author.*

An email address has been provided for each author.

*9. Keywords: Please remove commercial language (Tween20, Illumina). Please ensure that there are at least 6 keywords or phrases.*

Commercial language has been removed, there are 6 keywords or phrases.

*10. Summary: Please rephrase to clearly describe the protocol.*

The summary has been rewritten to more clearly describe the protocol.

*11. Please define all abbreviations before use.*

All abbreviations are now defined before use.

*12. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents. You may use the generic term followed by “(see table of materials)” to draw the readers’ attention to specific commercial names. Examples of commercial sounding language in your manuscript are: Tween20, Illumina, RosetteSep, STEMCELL Technologies Inc., cryotubes, Dynabeads, Invitrogen, Life Technologies, DynaMagnet, Qiagen MinElute, Agilent, etc.*

All commercial language has been removed from the manuscript and contained to the Table of Materials.

*13. 3.1.3: What is used to wash and what volume? Please specify.*

This step has been rewritten for clarity.

*14. 4.2.3.1: What is washed?*

This step has been rewritten for clarity.

*15. Please combine some of the shorter Protocol steps so that individual steps contain 2-3 actions and maximum of 4 sentences per step.*

The protocol steps have been reworked to combine shorter steps and provide more clarity.

*16. 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.*

Less than 2.75 pages of protocol has been highlighted for filmable content.

*17. Discussion: Please discuss any limitations of the technique.*

The current limitations of the protocol are now more clearly highlighted as a discussion on the necessity of re-optimizing the protocol if used with other cell types or cell volumes, and in different contexts, such as single cell RNA-seq.   
  
*Reviewer #1 Comments:  
  
In Figure 2, the large DNA fragment around 1000kb is not typical in ATAC-seq library and might be misleading. I suggest author to provide several more image to help readers have a better idea of how ATAC-seq library looks like.*

We agree that the large DNA fragment around 1000 kb is not typical in an Agilent BioAnalyzer trace of ATAC-seq libraries. However, the traces presented in this paper are from the Agilent TapeStation, and often show such a band at 1000 kb. This is because the sizing range of the High Sensitivity D1000 ScreenTape we use from TapeStation only go up to 1,000 bp, any DNA fragments bigger than 1000bp is compressed into the dark band that’s shown at 1,000 to 1,500bp (the upper marker). Thus this is due to the limitation of the TapeStation not the quality of our ATAC-seq libraries. All quality control of the samples presented in the paper was done with Agilent TapeStation. We also agree that more examples are warranted, and at the reviewer’s suggestion have provided two samples’ gel electropherograms and traces to give readers a better idea of what to expect from their ATAC-seq libraries.  *In Figure3, only one result from improved protocol are shown. As author did a bunch of experiments, it is necessary to show the results from several replicates to demonstrate the reproducibility of method.*

We have included a figure on biological replicates to demonstrate the reproducibility of the method.  *As several previous publications addressed this question in other ways, it is important to discuss and compare them in the discussion part (Wu, J. et al. 2016, Nature; Corces, MR. et al. 2017, Nature Method).*We thank the reviewer for reminding us of these papers and the methods they employed to address the question of mitochondrial contamination, and we have included them in our discussion.