Reviewer Report

Title: **A workflow for simplified analysis of ATAC-cap-seq data in R**

Version: **Original Submission** **Date:** 1/29/2018

Reviewer name: **Noboru Jo Sakabe**

Reviewer Comments to Author:

In this manuscript "A workflow for simplified analysis of ATAC-cap-seq data in R" by Shrestha et al, an R package (atacr) wrapping existing tools was created to analyze ATAC-cap-seq data. The package receives alignment data as well as bait and other sample information as input, creates an R object that encapsulates the experiment data, provides QC plots, performs normalization and differential read count analysis.1. atacr plots correlations between samples as a QC measure. It would be useful if a clustering or PCA plot was also provided so the user can more easily verify sample mismatches, effect of treatment and batch effects.2. The authors should clarify that the package doesn't allow for experimental designs more complex than control/treatment. For example, the edgeR exact test is only for single factor data.3. The authors should provide guidance to when the different normalization methods should be used.4. differential_windows.Rmd doesn't seem to have an example of how to use edgeR for differential window analysis. Is estimateDisp() used? edgeR was created with genome-wide data in mind, instead of data from a few sites. edgeR borrows information from other genes to estimate dispersion of read counts. With so few sites in an ATAC-cap-seq data set, this procedure is unlikely to make sense. The authors must explain how they are using edgeR and how they adapted it to analysis of a few sites.5. The package would benefit from a single tutorial like the ones existing for several R packages (e.g. the edgeR and DESeq2 vignettes), instead of several different files.6. The authors should include a real dataset with raw data, i.e. .bam and metadata files, especially for peer-review along with a single file tutorial with all the steps necessary to go from raw data to differential windows.7. pg 1, ln 30: I suggest avoiding phrasing that inverts the logical flow of thought ("upstream ATAC-seq step").8. pg 1, ln 55: The authors cite the original ATAC-seq paper for ATAC-cap-seq. Was this method published? Can the authors cite papers that used ATAC-cap-seq?9. How is the bait information used? Are windows stitched? Are non-baited windows used? Are only baited regions reported as differential? The authors should provide a comparison of their window-based method with standard peak callers and provide screenshots of the peaks and differential windows identified with the different methods.10. Recall and precision are swapped in Fig 2.11. Overall, I think the manuscript should better explain how atacr performs each step, including information in comments 2, 3, 4 and 9.12. Fig 1: control_003 and treatment_002 seem to have been swappped.

Level of Interest

Please indicate how interesting you found the manuscript: An article whose findings are important to those with closely related research interests

Quality of Written English

Please indicate the quality of language in the manuscript: Acceptable

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