Reviewer Report

Title: Comparative Analysis of common alignment tools for single cell RNA sequencing

Version: Revision 1   Date: 10/25/2021

Reviewer name: Bo Li, Ph.D.

Reviewer Comments to Author:

The authors did a very great job addressing my concerns. I can foresee this manuscript as a great benchmarking paper for the community. However, It is still important for the authors to clarify on the following questions before a publication:

1. In last paragraph of discussion, we have "If high-quality cell counts need to be obtained, Alevin appears to be the most suitable method, as average gene counts 23 are high- and poor-quality barcodes are seldom reported."

This statement is super confusing because in Figure 5, the authors recommended Alevin-Fry over Alevin for all cases? In addition, I do not see why if high-quality cell counts needed, Alevin is necessarily the best tool.

2. The last sentence in the same paragraph - "For very large projects with a high number of samples, pseudo-alignment tools such as Alevin-fry or Kallisto can be advantageous in terms of runtime and storage efficiency, at the cost of a slight reduction in accuracy."

This statement is not well-supported by the data. Based on Figure 1, the runtime of STARsolo and Alevin-fry are very similar. If the authors want to claim pseudoalignment methods are advantageous in terms of runtime, I think only Kallisto should be mentioned. However, the authors also raised concerns regarding Kallisto's accuracy. So in this case, I am not sure if it is "at the cost of a slight reduction in accuracy".

3. STARsolo also provide multi-mapping reads assignment:
https://github.com/alexdobin/STAR/blob/master/docs/STARsolo.md#multi-gene-reads. Please adjust Figure 5 accordingly.

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