

Reviewer Report

Title: Impact of reference design on estimating SARS-CoV-2 lineage abundances from wastewater sequencing data

Version: Original Submission **Date: 1/15/2024**

Reviewer name: Liuyang Zhao

Reviewer Comments to Author:

In this study, the authors initiate a novel exploration by employing parameter escalation experiments to assess the impact of reference size and alternative allele frequency cutoffs on the effects of virus lineage composition in wastewater samples and their references. The research provides valuable insights into how different parameter settings influence outcomes in test data sets, particularly highlighting the role of virus lineage composition in wastewater samples and the corresponding references. Detailed parameters for these analyses are made available in several bash files at osf.io/upbqj. Despite these significant contributions, certain areas could benefit from further enhancement:

1. The current methodology utilizes Ion Torrent for testing mock samples. However, this approach may not fully capture the variability in alignment and sub-lineage analysis. Incorporating additional sequencing data from PacBio, Nanopore, and Illumina would offer a more comprehensive examination of these aspects, potentially leading to more robust findings.
2. While the study showcases a variety of pipelines based on mutation-based and sequence-based tools in Table 1, the evaluation of three data sets was limited to only using MAMUSS (as a mutation-based reference) and VLQ-nf (as a sequence-based reference). For more conclusive guidance in pipeline selection, it is advisable for the authors to expand their analysis to include at least two or three more pipelines. This recommendation aligns with observations noted by the authors at line 619, suggesting a comprehensive benchmark comparison would significantly enhance the study's utility and appeal to readers seeking optimal pipeline strategies.

Methods

Are the methods appropriate to the aims of the study, are they well described, and are necessary controls included? Choose an item.

Conclusions

Are the conclusions adequately supported by the data shown? Choose an item.

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