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**DEPARTMENT OF MICROBIOLOGY AND IMMUNOLOGY**

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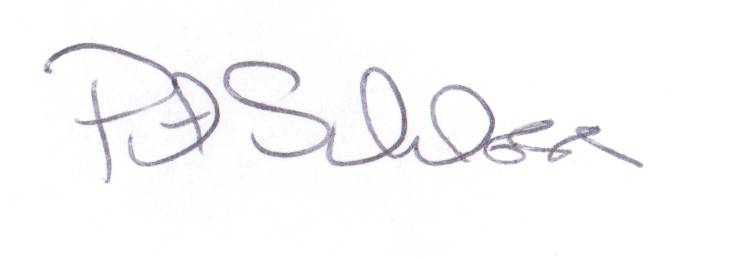


Dear Dr. Imperiale,

I am happy to submit my manuscript, **Waste not, want not: Revisiting the analysis that called into question the practice of rarefaction**, for your consideration at *mSphere*. Among studies sequencing 16S rRNA gene fragments, it is common to observe wide variation in the number of sequences sampled from each sample in the study. This manuscript readdresses a widely cited paper from 2014 that indicated that rarefying data and with it rarefaction, should not be used in amplicon sequencing studies. I have recreated their simulated framework and evaluated different analysis decisions from their study. In the end, using their framework, I show that rarefaction should be used. This manuscript has been posted to bioRxiv as a preprint (DOI: 10.1101/2023.06.23.546312).

I have also submitted another manuscript to *mSphere* that applies normalization approaches that are recommended in more modern studies using a more robust simulation framework (**Rarefaction is currently the best approach to control for uneven sequencing effort in amplicon sequence analyses**; DOI: 10.1101/2023.06.23.546313**)**. The conclusion from that manuscript is the same as this – rarefaction ought to be used to control for uneven sampling effort. Although these manuscripts touch on the same question, they use different approaches. I feel that both stand on their own as independent manuscripts.

Sincerely,



Patrick D. Schloss, PhD

Professor