**Materials & Methods**

The input data was sequenced from the ZymoBIOMICS™ Microbial Community DNA Standard, Catalog Nos. D6305 (200ng) and D6306 (2000ng). This kit contained a list of the different bacteria and fungi within, as well as their percent abundance. Long-read data was collected by using Oxford Nanopore Technologies (ONT) sequencing. Short-read data was collected by using Illumina sequencing. CIAN Diagnostics coordinated the sequencing and provided access to the data.

BugSeq was used to analyze long-read data. The version was listed as “latest” on their website, but should be whatever build follows their 7 November 2022 4.0 release. For the options used, Platform was set to Nanopore and Device & Chemistry was set to MinION/GridION/Flongle – R10.4/10.4.1. Within 6 hours I received an email with a link to online interactive results, which become unavailable after 30 days. However, I was able to download all of the results I needed. BugSeq did a quality control check and then classified both the raw reads I uploaded, then assembled them into longer contigs, and then classified those too. This provided me with both raw and assembled classification results.

Kraken2 was the first program planned to analyze short-read data. However, problems occurred right from the start. Kraken2 was installed through Conda, which grabbed the older version 2.0.7. instead of the latest version 2.1.2., and as a result tripped errors when trying to build the standard reference database. However, once the program was updated, the build command still did not work. At first, this seemed to be a security issue, as the FTP function that Kraken2 uses is considered insecure by our campus firewall. But even after allowing Kraken2 a way though, the command did not work. Only after our campus system administrator tweaked the “rsync\_from\_ncbi.pl” files was the reference database finally downloaded. However, the final command to classify data refused to run. This is likely because there was either a problem downloading the reference database or a lack of RAM. Reinstalling the database did not fix the problem. As of yet, I have been unable to get Kraken2 classification results.

CCMetagen was the second program planned to analyze short-read data. I used version 1.4.0. Since this program does not have a build-in command to install reference databases, I had to retrieve the standard database using wget. Initially, I was stymied by problems accessing our campus cluster. Once this was resolved, the file host appeared to block our server’s attempts to download the files. Our campus server administrator was able to download the files manually through his browser and get them on the server with WinSCP. This work around enabled me to build the reference database with no further problems. However, upon running the command to classify data, it tripped errors about not being able to allocate enough RAM. Swap space was increased to 31 GB but this did not solve the matter. As of yet, I have been unable to get CCMetagen classification results.

Centrifuge was the third program planned to analyze short-read data. I used version 4.8.2. Like Kraken2, it had errors from the start when trying to build the reference database. This was initially thought to be related to troubles with accessing our campus SLURM cluster, an issue that stalled progress with all three short-read programs. However, the error persisted after that was resolved. Ultimately, my efforts were redirected towards debugging Kraken2 and CCMetagen, as Centrifuge was a program CIAN Diagnostics had already tried and found unimpressive.

Comparison metrics were quantified by the abundance estimates present in the kreport files. Where the report estimates for a species overlapped with the original kit, the percentage was marked as a true positive. Where the estimate of a species exceeded those in the kit, the excess was marked as false positive. Where the estimate of a species was less than those in the kit, the difference was marked as a false negative, and the false positive amount was subtracted to prevent it from being counted twice. From there, precision, accuracy, F1, and F0.5 were calculated.