

We've upgraded our Microbial Diversity Profiling Pipeline to the NextSeq™ 2000.

What is the microbial profiling pipeline?

The microbial profiling pipeline aims to study the composition and diversity of microbial communities using marker gene sequences. The computational workflow includes quality control, data processing and taxonomic assignment.

Why have you changed your pipeline?

With the transition of the microbial profiling workflow to Illumina NextSeq™ 2000 from MiSeq, we can now introduce a highly anticipated feature during the data processing: Paired-end read support. This new capability is integrated to leverage the high quality of short-read sequences obtained from the NextSeq 2000 platform.

Due to previous limitations of the MiSeq platform, the analysis relied on single-end reads for microbial profiling resulting in the use of only about 250bp high-quality reads for taxonomic classification. Paired-end reads from the NextSeq 2000 provide overlapping sequence information, enabling longer reads for the taxonomic assignment.

The delivered output remains consistent with the recent microbial profiling pipeline. The delivery includes bar charts and Excel files which provide an overview of the microbial composition.

We have incorporated an additional step to merge the reads prior to forming ASVs. Furthermore, the transition to NextSeq 2000 required us to use different error models and quality control metrics for trimming the reads.

What else have you changed with the pipeline?

As part of our updates, we have included a default step to remove Chloroplast and Mitochondria from the analysis. Upon request (additional charges will apply) we can also provide further services such as the calculation of alpha and beta diversity and PICRUST analysis which provides the functional predictions of the microbial community.

Why do I have two fastq files for each sample in two different folders?

NextSeq 2000 generates a much higher number of reads than our guarantee. The pipeline's parameters are specifically optimised for a sample size of 50,000 sequences. This particular size was selected to ensure a quick turnaround time while still encompassing a sufficient number of sequences to effectively capture the microbial composition. As part of our microbial profiling service, we provide both the raw data and the data utilised for the analysis.

MICROBIAL DIVERSITY PROFILING 2.1 FAQs



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This all sounds great, but what if I've completed my part of the work using MiSeq?

We'll still maintain our Divpro 2 pipeline and the MiSeq upon request. If you specifically require MiSeq analysis for an ongoing project, please contact your Account Manager and discuss your options.

Why can't I see html plots, when I double click files after download?

By default, the folders are zipped while downloading results. When you double-click on the file, Windows OS by default will show the content of the folder, but you still need to "unzip" files in order to view or use them.

To unzip the files:

1. Hover your mouse over the zipped file and right-click.
2. Select "7z" (or any equivalent software).
3. Select "Extract files" (or) "Extract to folder".

Then use the extracted folder for viewing html plots.

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