

## Accuracy is key: how to unlock the most from your microbiome samples

Once samples are collected and your library is prepared, it's time for the main event. But when it comes to sequencing of microbiome samples, how can you ensure accurate data? We sat down with Dr Nicola Angel, Head of Laboratory Operations at Microba Life Sciences, to hear her advice for getting the best sequencing results.

Microba is a precision gut microbiome analysis company that is using insights from the human gut to drive new diagnostic and therapeutic discoveries. They know that to transform data from the human microbiome into effective health solutions, accurate and reliable sequencing is key. Let's hear some words of advice from Dr Angel.

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## Getting ready for sequencing

“Preparation is critical to ensure accurate sequencing results,” says Dr Angel. She notes that it is important to establish foundational processes early in the operations: is there enough stock from one batch of reagents to process your samples? Are the kits ready and have the reagents been mixed well prior to use? It is also important to collect and store samples using reliable methods that preserve the original microbial content. Plan your workflow to minimize degradation of your samples; for example, multiple freeze-thaw cycles can impact the integrity of the DNA, and this will have a significant impact on the output of both your library and final microbiome analysis.

Another essential part of the planning process is to select a library prep method that will support the required data analysis. Make sure you are using an accurate, reproducible method that is compatible with your type of sample.

“People sometimes skip these basic steps, and it definitely impacts the quality of your data,” she adds. “Have checklists and don’t rush things. Once you cover your bases, Illumina’s robust sequencing processes offer a great option for researchers with any level of experience.”

## Choosing your Illumina sequencer

We asked Dr Angel to share her top choice when it comes to sequencing equipment. “At Microba, we have used practically the whole Illumina fleet, but we really love working with the NovaSeq™ 6000 for sequencing all our gut microbiome samples. Our team has completed over 600 sequencing runs on this instrument—it is very reliable, flexible, and user friendly,” explains Dr Angel.

“We routinely use a variety of different workflows, which allow us to run anywhere between 48 and 6,000 samples on a single run. However, the selection of run format will differ based on the complexity of the sample you’re analyzing; for complex microbial communities, such as soil or gut microbiome samples, we recommend around 100 samples per S1 flow cell. This will provide an optimal amount of data to detect lower abundance microorganisms, perform genome mining, or analyze the functional potential of the sample.”



## Determine your project needs

The Illumina range of sequencing products are designed to support diverse applications and sample types. Dr Angel describes what sequencing parameters she would recommend for those just starting out in microbiome analysis.

“Your sequencing depth depends largely on what you want out of your analysis—for example, if you want strain-level resolution or are looking at specific genomic regions, such as antimicrobial resistance genes, you may need up to 16 million reads. For less complex samples, two or three million reads works well. For gut microbiome samples, we find that around seven million paired-end reads is a nice place to start, and you can modify up or down as needed,” she explains.

“As for read length, longer paired reads are preferable. Longer reads result in overlap regions, which allow you to monitor the library insert size, and the increased genome coverage will be helpful when you go to map those reads.”

## The importance of controls

Controls are critical for ensuring confidence in your sequencing data. And Dr Angel takes pride in her reputation for going overboard when it comes to controls. “We use positive and negative controls at each step of the workflow, from DNA extraction through to sequencing,” she explains.

Effective analysis of the sequenced controls will help you monitor and improve sequencing outcomes. Where possible, automating these processing pipelines allows your team to quickly check the quality of your library prep and sequencing data. For those who are new to microbiome sequencing, you can purchase controls through commercial vendors.

In addition to routine positive and negative controls, allowing for batch controls is important. “If we are doing the sequencing for a large research project, we like to repeat a sample from a previous set on the next run so that the researcher can be confident that no changes were introduced during the sequencing process,” Dr Angel adds.

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## What metrics are important for QC?

Once you have access to good quality positive and negative controls, what should you actually be looking for to know if you have accurate data?

“We maintain a register that we add every single run to. Our analysis of controls typically includes checks for contamination, quality of sequences, accuracy of reporting known content, and comparison to previously sequenced samples. This allows us to track metrics over time. Illumina suggests the [PhiX control library](#), which provides a reliable internal control for sequencing and read alignment—it’s well established and includes some great metrics that are automatically generated for review,” Dr Angel explains.

“When assessing gut microbiome samples, we like to look at the %Q30 scores. This reports on the percentage of bases with a quality score of over 30, which is considered ~99.9% accurate.” **Read more about Q scores [here](#).** “The %Q30 score is an excellent metric to evaluate a run. We may also look at yield, which is the number of bases that passed filter, and the error rate, which can be calculated using the PhiX alignment. We also like to see fairly even coverage of each base captured during sequencing, or the percent base score. These kinds of metrics can tell whether you should have confidence to proceed to report generation.”

While analyzing microbiome sequencing data can often require the specialist skills of a bioinformatics team, there are tools to help your laboratory team quickly check the quality of a run. “We highly recommend using the [BaseSpace™ Sequence Hub](#). This is a great intuitive platform that stores run data and allows you to make quick comparisons over time.

This gives you a lot of flexibility in how much detail you would like to review and can help catch major issues early on without spending time on a full analysis,” explains Dr Angel.

“Finally, we have found tools like a Laboratory Information Management System (LIMS) and Illumina’s [Experiment Manager](#) to be really helpful for sample tracking and automating workflows.”

## Final thoughts to help you uncover more from your samples

Accurate sequencing is critical when exploring the taxonomic and functional diversity of microbial communities. You want to make sure the instrument you choose can be tailored to your study needs, while providing a simplified workflow to increase the efficiency in your laboratory and minimize errors.

A final piece of advice from Dr Angel? “Make sure you look at front end processes: are your samples collected appropriately? Is your DNA stored and extracted properly? Do you have a high-quality library prep? Once the front end is taken care of, Illumina sequencers will make the rest easy.”

Learn more about the Illumina sequencing range [here](#).

## Illumina & Microba: Empowering microbiome research

Microba Life Sciences and Illumina work together to accelerate microbiome research. Combining Microba’s high-quality proprietary gut microbiome [Analysis Platform](#) with Illumina’s revolutionary [Next Generation Sequencing](#) tools, researchers have access to world-leading, accurate metagenomic data to drive new discovery from the microbiome.



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