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Top tips for optimizing NGS library preparation for microbiome analysis

Next-generation sequencing (NGS) has transformed our ability to study the role of microbial communities in human, animal, and environmental health. But in order to get accurate and meaningful results, how you prepare your samples for sequencing is critical. This includes choosing the right library prep method for your samples.

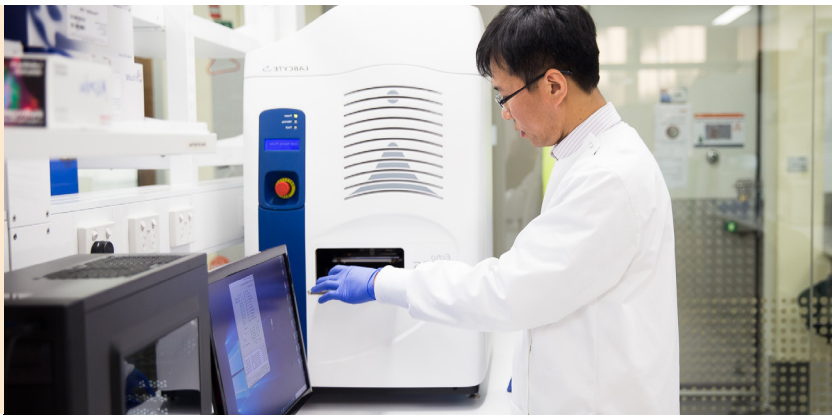
What is library prep?

Before sequencing can begin, genomic DNA must be converted into a form that is compatible for use with an NGS system. This involves converting the DNA into similarly sized fragments. Unique index and sequencing adapters are then added to both ends, allowing many libraries to be pooled for sequencing. Each step of library prep plays an important role in producing high-quality NGS results.

So, how can you optimize your library prep? We put together our top tips for getting the best sequencing results.

1.

Pick a library prep kit with demonstrated performance for your sample type



When extracting DNA from your primary samples, it is important to use recommended reagents that are designed for microbiome samples to minimize extraction bias. The same consideration applies to library prep – you want to make sure that the kit you use is the best fit for the type of sample you are analyzing. Kits or protocols that perform robustly over a wide range of different sample types, such as the Illumina DNA prep kit, are convenient for busy laboratories or small research teams that may need to efficiently cover a variety of projects. However, the best practice is to determine an approach with demonstrated quality and reproducibility in your sample type at the start of your research project.

You also want a flexible product that allows you to work with a range of DNA concentrations. For sequencing small microbial genomes, quality libraries can be generated using as little as one ng of input DNA.

The Illumina DNA Prep kit supports a broad DNA input range (1–500 ng), so it's well suited for processing microbiome samples.

We asked Dr Nicola Angel, Head of Laboratory Operations at the precision gut microbiome analysis company, Microba Life Sciences, what type of kit she prefers to work with.

"When we sought out to establish our large human gut microbiome database, we did a lot of benchmarking of different library prep products to ensure we were getting the most reproducible, reliable and accurate results," explains Dr Angel. "We found that the Illumina DNA Prep kit performed best for this type of sample; it reports the microorganisms of the gut microbiome very accurately, and as a bonus, it has a really nice workflow."

2.

Enhance your ease of workflow and improve reproducibility

Speaking of workflow... library prep has multiple steps, including DNA fragmentation, library preparation and library normalization. As such, many laboratories consider library prep to be a bottleneck in the NGS workflow. Optimizing your library prep workflow can help improve NGS throughput and scalability and promote consistency in your results.

With the Illumina DNA Prep kit, the on-bead "tagmentation" process, which combines the fragmentation and adaptor ligation steps, reduces the total library prep time to 3–4 hours for maximum efficiency. This integrated approach is shown to generate consistent and uniform insert sizes. If your sample has 100–500 ng DNA available, the Illumina DNA prep kit eliminates the need for normalization steps such as library quantitation before pooling and sequencing.

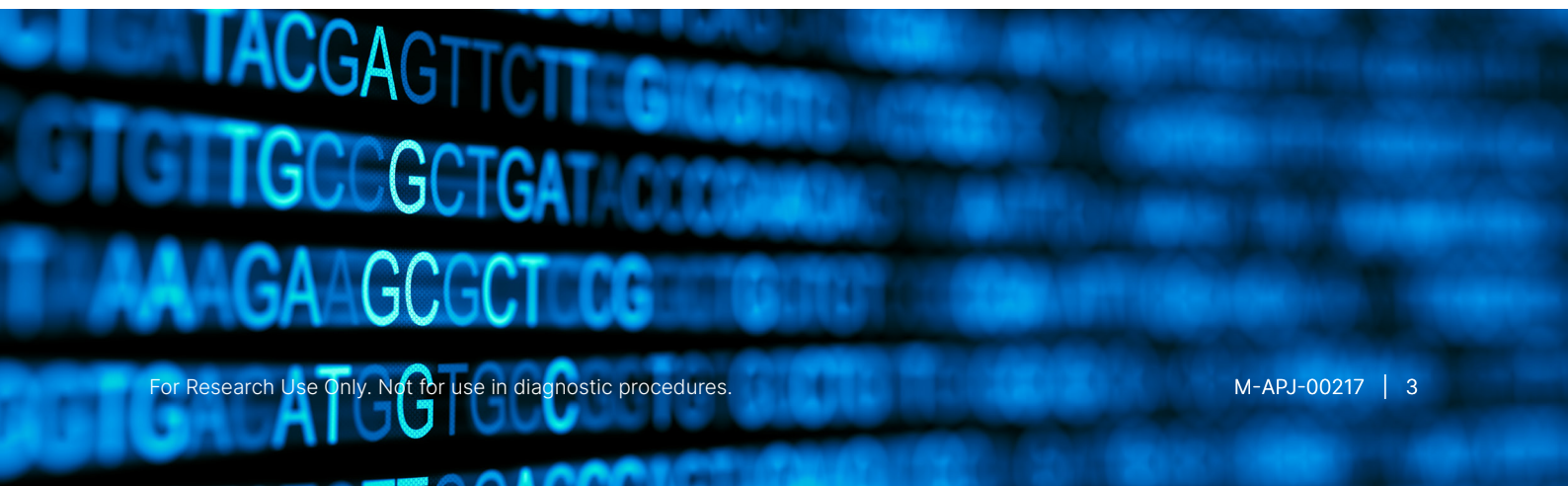
When establishing your NGS workflow, look for a library prep kit that makes use of the equipment and staff that are already available in your laboratory. You want to consider aspects such as pipetting volumes, number of pipetting steps, complexity of the protocol, and time required. A workflow that is user-friendly and that supports users of all experience levels can also help reduce the risk of introducing human error.

3.

To improve output, consider using unique dual indexing for multiplexing

Multiplexing allows you to pool and sequence large numbers of libraries during a single run, thereby increasing the number of samples you can sequence without drastically increasing the cost or run time. This is particularly useful when working with small genomes (including microbial samples) or targeting regions of interest.

During library prep, a unique identifier (index) will be added to DNA fragments so that the sample can be later identified. When using a library prep method that supports unique dual indexing, each column has completely unique indexes, so a 96-well plate can support 96 dual indexed libraries. Illumina also offers 384 dual index options that can be used when preparing samples to further enhance the multiplexing capacity. Compared with combinatorial dual indexing, unique dual indexing reduces the frequency of misassigned reads, thereby improving the sensitivity of your run. This can help improve your laboratory's sequencing performance and throughput.



4.

Design your workflow to minimize error and increase reproducibility

If you are working with only a few samples, manual library prep may be a good option. However, if you have many samples to process, automation can improve your library prep workflow by eliminating the need for lengthy, labour-intensive sample handling while also reducing the risk of human error or contamination. A flexible kit that can accommodate both approaches will allow you to tailor your library prep to meet your specific project needs. Early on in establishing your workflow, check the data to show that automation of the platform is providing high-quality, accurate libraries. You should always include a QC step in the automated workflow so you can see if something goes wrong.

Dr Angel notes that her lab exclusively uses automation. “It comes down to reproducibility. There’s no doubt that there is less error introduced with automated workflows compared with manual pipetting or handling multiple samples. Even if you have all the correct processes in place, things can still go wrong. Automation helps minimize human error while also significantly increasing the efficiency of your library prep.”

“Another benefit of automation is traceability,” Dr Angel adds. “You can very clearly see from the instrument logs what errors might have occurred in the process.”

Automation can help improve reproducibility, traceability, increased throughput, and efficiency, while reducing the per-sample cost. The Illumina DNA Prep kit is automation friendly and there are Illumina Qualified scripts available for most liquid handling platforms, ensuring a smooth transition to automation.

5.

Never neglect controls and QC during library prep

Using appropriate controls and QC processes to evaluate your NGS workflow quality can help set you up for successful sequencing outcomes. To do this, ensure you have positive and negative controls for each step of the process, including DNA extraction and library prep. This allows you to audit your sample processing and make corrections if anything goes wrong.

You can optimize and validate your DNA extraction and subsequent library prep using well-defined controls. There are a range of commercially available Microbial-Community-Standards, which are comprehensively characterized mock communities that you can use to evaluate the quality of your NGS workflow. Make sure to use duplicates or triplicates of these controls to assess repeatability.

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What type of library prep is right for you?

Next-generation sequencing has accelerated discoveries in microbiome research. But to get the most out of your data, you need to set yourself up for success with a quality library prep. It is important to optimize your library prep workflow so it is fast, flexible, reproducible and, most importantly, accurate.

Learn more about how the Illumina DNA prep kit can help improve the efficiency and quality of your libraries 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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