

NGS Sample Preparation: Tips from Five Experts

Next-generation sequencing (NGS) has allowed researchers to obtain genetic information with more speed and efficiency than ever before. When it comes to NGS, the key to obtaining high-quality results lies in well-executed sample preparation procedures. We asked five NGS experts to weigh in on how to improve sample preparation processes for next-generation sequencing and this is the advice they gave:



The main thing to keep an eye on is the **quality of your input**. Regardless of how well you are performing your library prep, the results will always only be as good as your input material. In addition, you should always make sure to stay inside the specs of the library prep kit. Most kits work above or below the recommended specs, however the success of such an experiment will always be a bit difficult. Finally, **experiments should be set up in triplicates** or more in order to distinguish any eventual outliers.

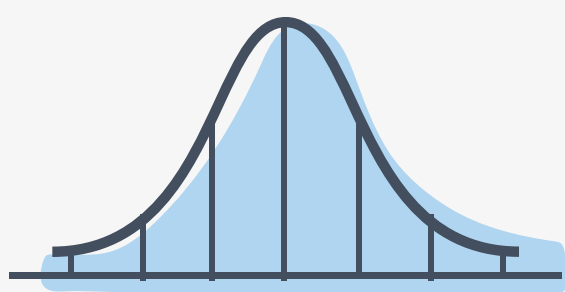
—Ferris Jung, Research Technician at EMBL

Nucleic acid quality is more important than ever. Choose a purification method that produces not just a large quantity of DNA or RNA, but one that **generates material that is actually usable**. Take the time and effort to properly **QC and quantitate** your sample to minimize wasted sequencing and poor results.



—Kurtis Knox, Senior Strategic Portfolio Manager at Promega

To get the most out of **long read technologies**, sample prep is an important component. Many approaches focus on streamlining workflows and decreasing input amount but the key steps to ensure success are:

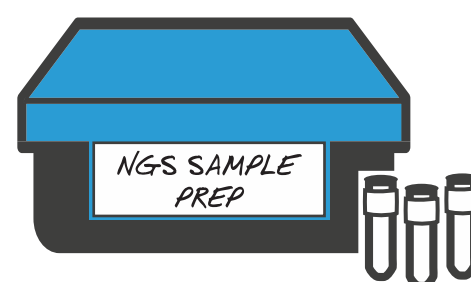


- **Ensure you have accurate mass and size distribution of both the incoming sample and final library.** For incoming sample QC perspective, it is essential to understand the amount of dsDNA and size distribution to ensure that you have sufficient DNA to complete your project and the size distribution will be long enough to ensure the quality of your final research. Accurately characterizing these same metrics on the final library is required to confirm the number of active polymerase binding sites. If not measured accurately, you will either add too much or too little sequencing polymerase. The former will likely add background signal and the latter may lead to low loading.
- Discuss your experimental design with a **trained expert**. Long read technologies and associated technologies are constantly improving and evolving. Getting the latest recommendations on gDNA extraction, quantification, size determination, and size selection can be very informative.

—Paul Kotturi, Director of Product Management at PacBio

Select the right library prep kit and panel based on your needs. Factors that need to be taken into account include application, the sample type, the DNA input type and amount, the sequencing platform, and the capacity.

- For samples with **limited material** such as a needle or liquid biopsy, you want to select a product that requires less DNA input with higher conversion efficiency.
- If you plan to use a **small desktop sequencer** to do a large cohort study, panel size and the ability to customize to only include genes of interest may be important.
- Important QC metrics such as the overall DNA concentration, DNA quantity, amplifiable DNA quantity, and DNA fragment length distribution are important to ensure accurate results, low failure rates, and cost-effectiveness.
- If you have a **high capacity sequencer**, you would want to choose a library prep solution with expanded sample indexing options, so more samples can be run together.
- If you work on **somatic applications to detect very low-frequency mutations**, you would want to have a sample prep solution including analysis with error correction capabilities using molecular barcode.
- Lastly, **mechanical vs. enzymatic DNA fragmentation**, FFPE compatibility, hands-on time, overall turn-around-time, and reagent cost are other things worth considering when selecting your library prep kit and panel.



—Viresh Patel, Ph.D., Senior Director of Marketing, Genomics, Diagnostics and Genomics Group, Agilent Technologies



The most important aspects of sample preparation for NGS are **knowing or determining the quality and integrity of your starting DNA** and **understanding the steps of the library construction process** that optimize either the sequencing efficiency or analysis type.

—Alex Vira, VP of Marketing at Sage Science