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Bridging the Efficiency and Throughput Gap with Magnis Automated NGS Library Preparation Solution

Introduction

Over the last decade, target enrichment has enabled researchers and clinicians to use next-generation sequencing (NGS) in a staggering variety of research areas and applications. Whether the research aim is whole-exome sequencing, or a focused small panel of genes, targeted sequencing can be used to quickly and easily identify variants of interest across a broad range of sample types.

Thanks to recent improvements in sensitivity, researchers can now use these same methods to study DNA from heterogeneous tumor samples and cell-free DNA samples to identify low-frequency variants of single-nucleotide polymorphisms (SNPs) and indels, and to resolve copy number variants (CNVs) and translocations. However, targeted NGS workflows often have lengthy processing and require significant hands-on time. With current technology, the library preparation process (which includes target enrichment) remains the most labor-intensive part of the workflow.

Automated vs. Manual Library Preparation

As the utility of target enrichment has expanded, many laboratories have begun looking for ways to streamline their DNA library preparation protocols to increase throughput and improve efficiency while maintaining library quality. Some laboratories have adopted automated protocols involving liquid handling systems. Other laboratories, however, continue with manual processing of DNA samples. Both methods have their advantages as well as their shortcomings.

Here, we will first explain and compare these two approaches, then introduce the Agilent Magnis NGS Prep system as a solution that meets the demand for increased productivity and lab operation-efficiency without sacrificing flexibility or quality. Due to its automated capabilities and ability to operate unattended enables labor efficient scalability for multi-run schedules. The Magnis system is uniquely suited for research and clinical laboratories that sequence several hundred to several thousand samples per year.

Manual library preparation

Manually processing of DNA samples for target enrichment and library preparation remains a common approach due to its ease of adoption and implementation. However, it requires high amount of hands-on time, which can create multiple challenges. When processing large numbers of samples, laboratories that rely on manual library preparation often struggle with throughput, lab efficiency, turnaround time, and library quality issues.

– **Lab efficiency:** each library preparation requires the time and attention of a trained technician or researcher. On average this involves several hours of hands-on time to process a batch of eight samples. As an example, consider a genetic research laboratory that manually processes one batch of eight samples each week for exome sequencing using SureSelect library preparation and target enrichment reagents. In this scenario, the technician's hands-on time adds up to 150 hours per year and, given the average hourly rate of a well-trained technician, this translates to an annual labor cost of several thousand dollars. If the lab runs multiple batches each week, then the associated labor cost will increase accordingly. Many laboratories would benefit from finding ways to make more effective use of their technicians' time.

– **Turnaround time:** a manual protocol typically needs around 10 hours to process extracted gDNA into a sequencing-ready library. Due to this requirement, the technician needs to allow enough time to either finish the library preparation protocol or get the samples to a safe stopping point before the end of the day. Because these limitations hinder flexibility, they ultimately increase the average turnaround time for generating sequencing ready libraries.

– **Library quality:** each step of a manual protocol introduces an opportunity for errors such as adding a wrong reagent, contaminating a sample, or mislabeling a plate. Becoming proficient at library preparation takes time and practice and even experienced technicians occasionally mishandle a step. When such errors inevitably happen, they incur costs associated with the additional labor and reagents needed to repeat that sample.

However, manual library preparation remains a viable choice in specific settings, such as low-throughput labs or those that are just starting to use NGS.

Automated library preparation

In many laboratories that have transitioned to automated library preparation, a liquid handling system performs the automated steps. These versatile instruments can process multiple sample plates at one time. They are able to maneuver plates, pipette reagents, incubate samples at a set temperature, and/or shake samples when they require mixing or aeration. The hardware and software is often highly customizable, versatile, and able to be used for a variety of non-NGS applications including affinity purification, qPCR, and cell-based assays.

Increased throughput, lab efficiency and consistent performance are the primary reasons laboratories choose automated library preparation. Once a satisfactory protocol has been established, laboratories can process several hundred samples per week with drastically reduced hands-on time.

An important consideration, however, is that most automated platforms are not completely hands-off and technicians cannot walk away from the system for the entire run. For most liquid handling automation platforms, NGS protocols still require a significant amount of time for reagent preparation and plating. Additionally, a technician must load those reagents onto the instrument at specific points during the protocol. Finally, if the automated liquid handling platform does not include an onboard necessary apparatus (such as a thermal cycler) then the technician must manually transfer the plates between the liquid handler and the apparatus.

When does automation make sense?

For laboratories that process large numbers of samples, automated solutions offer the potential for significant time and cost savings via high-throughput library preparation. However, most low- to medium-throughput laboratories (e.g., processing up to several dozen samples per week) need to carefully consider whether automation is an economical investment. Due to the upfront cost of high-throughput automation systems, these laboratories may not save enough on labor costs to recoup the initial expense.

Additionally, low- and medium-throughput laboratories may find that their reagent consumption per sample is higher when processing small batches (eight or fewer samples) on a liquid handler due, in part, to the dead volume of each reagent. This may drive up the per-sample reagent costs, particularly for valuable stocks of target capture oligo libraries and enzymes. However, if laboratories wait to process the samples in their queue until they have enough samples for a full 96-well plate, then they ultimately hurt their turnaround time.

An example of a medium-throughput laboratory would be a molecular pathology lab that analyzes up to a couple of thousand samples per year with multiple panels ranging from a small cancer panel to a comprehensive genomic profiling panel. During some weeks of the year, the lab may need to run several batches to meet needed turnaround times. While that level of throughput requires significant hands-on time for manual processing, it is too few samples to make an automated liquid handling system a cost-effective choice. In addition to the capital needed to invest in a liquid handler, this laboratory would see their reagent costs per sample increase considerably.

Another pain point for NGS laboratories that transition to an automated approach is the time spent optimizing manual protocols on their new automated system. Protocol optimization and repeated testing may be needed to develop an automated library preparation program that works as well as the manual protocol.

Due to the purchase price, inefficient reagent usage with small batches, and necessary protocol optimization, automated library preparation may only be ideally suited to laboratories that routinely process dozens of samples with each run. Such high-throughput labs include core facilities and other large laboratories that process one hundred samples or more per week can use a liquid handler for various applications.

The Magnis NGS Prep System

Different laboratories have different needs, but for many laboratories—such as those that process several hundred to several thousand samples every year — manual processing is still too inefficient. At the same time, automation with a large liquid handling platform is not cost-effective given their typical workload.

For these laboratories, Agilent offers a solution with the Magnis NGS Prep system. The Magnis system is an automated liquid handling system consisting of an instrument, pre-plated reagents, software, and pre-configured protocols optimized for library preparation and target enrichment. It can run a batch of eight samples and is able to perform all the steps of library preparation and target enrichment to produce sequencing-ready libraries for Illumina sequencers. The starting material for a protocol run is 10 to 200 ng of sheared gDNA isolated from blood, freshly frozen tissue, or FFPE samples.

The instrument includes all the necessary equipment and makes the Magnis system a completely enclosed, fully walk-away operating environment. Only five minutes of hands-on time is needed when setting up the instrument run and loading reagents and consumables onto the instrument's deck. From there, the Magnis system delivers eight libraries in less than nine hours without further operator intervention.

Advantages of the Magnis NGS Prep system

As an alternative to a traditional liquid handling automation platform or manual workflow, the Magnis NGS Prep System offers several advantages in lab efficiency, productivity, and performance:

– **Convenient run setup**
The library prep reagents for the Magnis NGS Prep System come pre-plated and require no further aliquoting. Additionally, because the protocol is preloaded into the Magnis system, it only takes five minutes for the technician to load the preplated reagents and consumables on the instrument and follow the intuitive touchscreen prompts to initiate the run.

– **Complete walkaway automation**
Unlike general-purpose liquid handling automation systems, once the technician completes the setup on the Magnis and starts a run, no further interaction is needed until the run is complete and the libraries are ready to be collected.
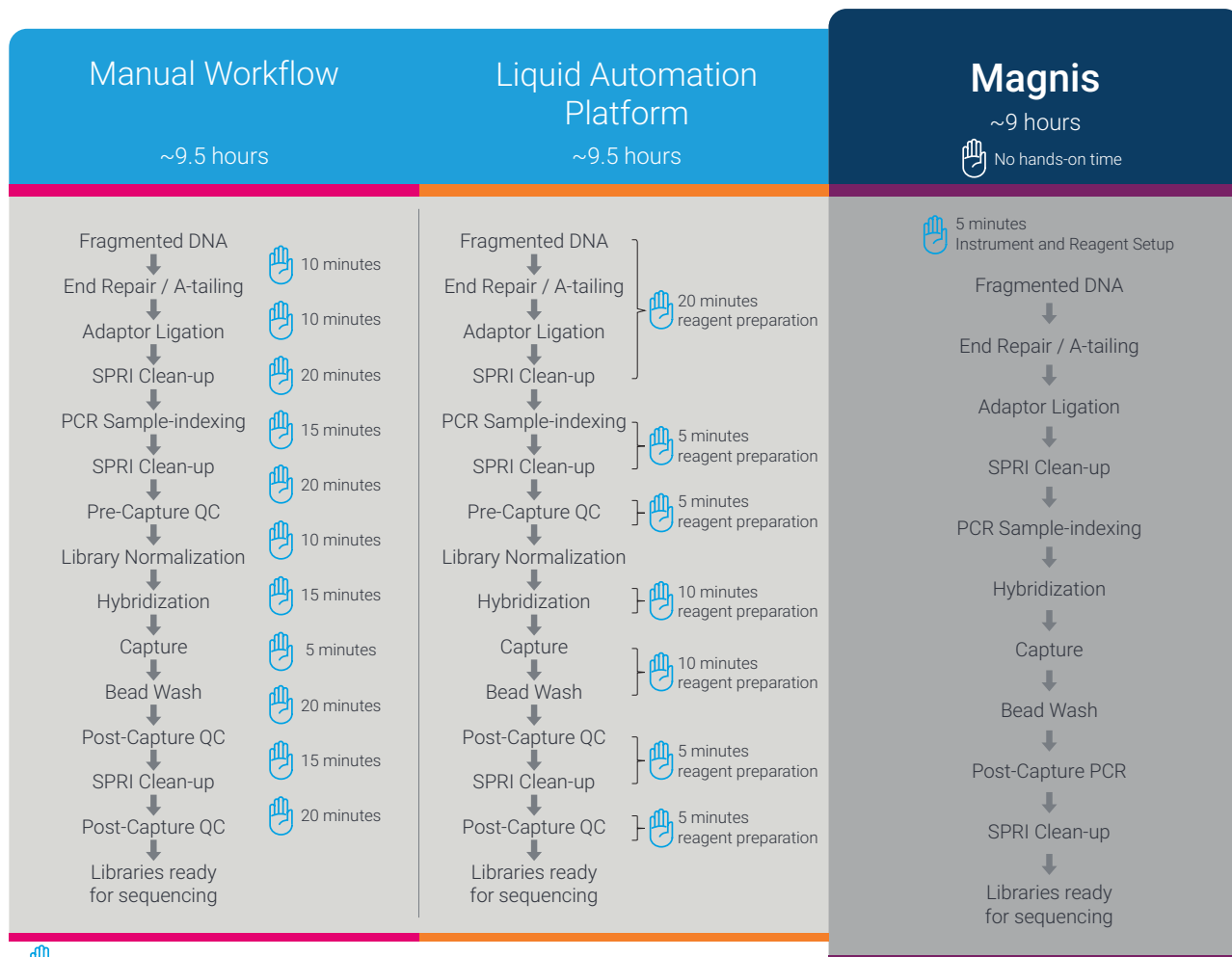
Figure 1. Workflow comparison for manual, liquid handling automation platform, and Magnis-based approaches. The hands-on time and overall turnaround time of the workflows are based on a batch of 8 reactions.

– **Cost-effectiveness**
  The Magnis instrument is only a fraction of the cost of a full-scale, multi-purpose liquid handling system. The technician labor cost for library prep and target enrichment is dramatically reduced with the Magnis system. To process a batch of eight samples using SureSelect XT HS library preparation and target enrichment, the hands-on labor time is 2.5 hours for the manual process, roughly an hour for a liquid handling automation system, and only five minutes on the Magnis system.

  The operating cost, including instrument cost and labor cost of re-running the NGS library preparation and target enrichment workflow, is one of the critical considerations for labs implementing routine targeted NGS workflows. For labs running routine targeted sequencing applications up to 2,000 reactions per year, the operating cost of the Magnis system is lower than either manual processing or an automated liquid handler, particularly for the labs whose labor cost of experienced technician is high.

– **Ability to accommodate diverse throughput needs**
  Liquid handlers are optimized for processing large batches of samples. These systems require that each run include at least a few dozen samples (optimally 24 to 96 samples) to use the reagents efficiently. If a laboratory does not routinely analyze samples at that pace, they may end up either consuming excess reagents to run smaller batches or hurting their turnaround time by waiting to group a sufficient number of samples in the queue.

The Magnis system, however, is designed to process eight samples per run and can accommodate two runs per day, making it the more efficient choice for many laboratories. Even large laboratories that already have a liquid handling automation system running NGS library preparation protocols may benefit from using a Magnis system to quickly process small batches of samples, particularly in the case of urgent samples and rerun failed samples that require a quick turnaround time.

– **Turnaround time**
  Processing eight libraries on the Magnis system takes less than nine hours and, because of its complete walkaway automation, technicians can start a run at any time of day. Coupled with a suitable downstream sequencing platform, laboratories can routinely achieve a three-day turnaround time from a submitted biological sample to a final report (Figure 2). Moreover, if the lab runs an extended shift, it can complete two consecutive Magnis runs in a single day, allowing them to deliver reports for 16 samples at the end of day three.
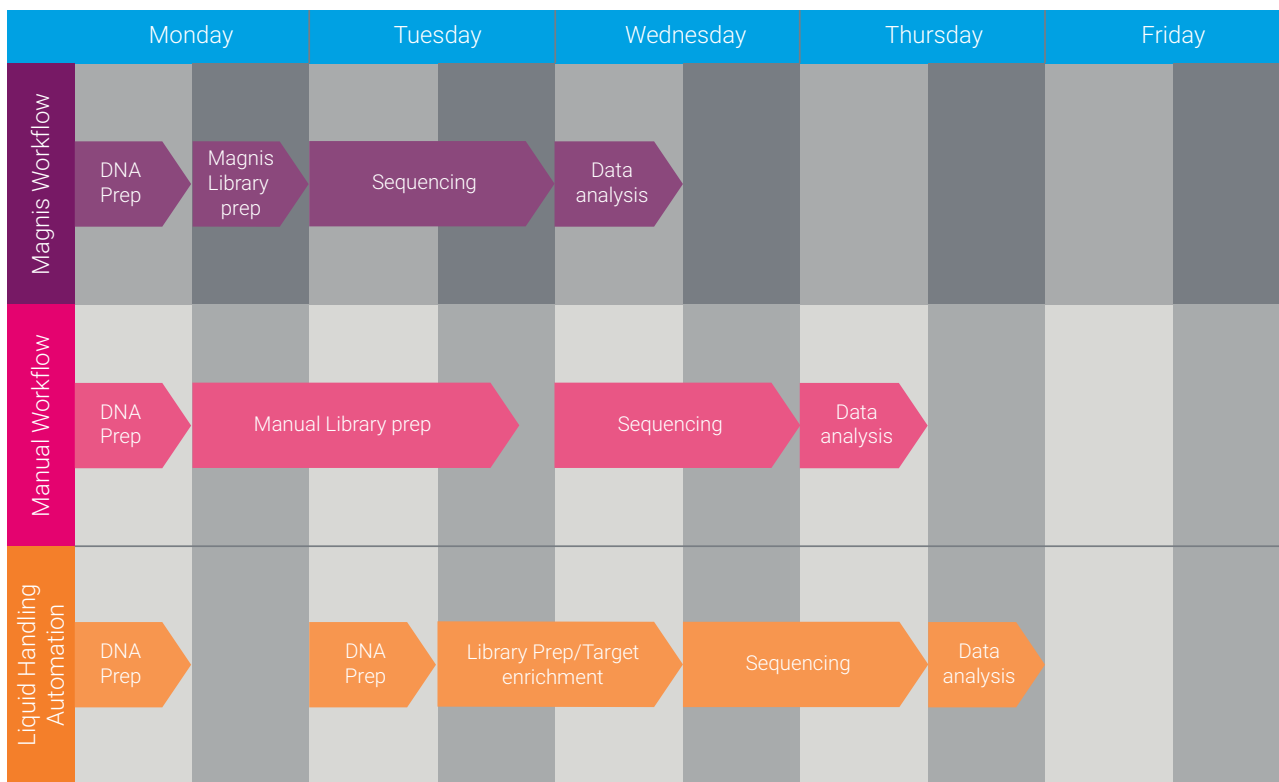
Figure 2. Overall NGS Workflow scheduling between Magnis, manual, and general liquid handling automation platforms. A liquid handling automation system may need a few days to batch up the samples to be cost-efficient (reflected in the above figure).

– **Reliability**

The Magnis system was designed specifically for NGS applications. As a result, the rate of sample dropouts and run failures on the Magnis system is extremely low, minimizing the number of repeat runs. This helps save operator time and reagent cost, meet turnaround time goals, and help researchers avoid situations where there is insufficient sample (such as an FFPE sample from a solid tumor) to repeat the workflow.

– **Improved reproducibility**

The Magnis system offers reproducibility comparable to manual processing by experienced operators (Figure 3).

Like the reagents and instrument, the Magnis protocols are developed and optimized for the industry-leading SureSelect library preparation and target enrichment technology. The first protocol released on the Magnis system, SureSelectXT HS-Illumina, is supported by SureSelect XT HS chemistry and has been extensively tested and validated with different biological sample types. Figure 3 illustrates that the Magnis performance is both reproducible and comparable to a manual workflow performed by an experienced NGS technician. With more Agilent-validated protocols becoming available in the near future, Magnis users will be able to quickly expand their applications by downloading new protocols directly to the instrument.

Figure 3. Quality of libraries generated by Magnis compared with those by manual prep. 10 ng of gDNA was extracted from both mildly and severely compromised FFPE samples, mechanically sheared, and used in each of the five Magnis runs with a 118 kb custom NGS panel. Sequencing metrics were compared head to head between these two methods for each of the four categories.

Conclusion

The Magnis NGS Prep System provides a balanced alternative to the two conventional approaches (manual processing and automated liquid handling) for NGS library preparation. The ability to walkaway, consistent performance, excellent library preparation performance, and cost effectiveness makes it an ideal option for any low- to medium-throughput NGS laboratory that uses target enrichment for genetic research, clinical diagnostics, or other NGS-based applications.

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