

Buyer's Guide:

Automated Library Preparation for Next-Generation Sequencing

What to consider as you evaluate options for automating library preparation.

Yes, success can be automated.

Next-generation sequencing (NGS) has propelled biological research to new heights, overcoming limitations in throughput and speed to ignite a revolution in genomic science.

Today's wide spectrum of NGS instruments makes the technology accessible for labs of different sizes. Sequencers are more powerful too, capable of producing hundreds to thousands of gigabases of data in a single sequencing run. From cancer and microbiology to forensics and agriculture, NGS is unearthing new knowledge that redefines how we see and understand the world around us.

But every successful sequencing run requires an equally successful preparation of DNA or RNA libraries. Each library must be carefully prepared according to the requirements of your application. Depending on which method is used to prepare libraries, this can be a time-consuming and resource-intensive process. If one step goes wrong or is forgotten, the entire run is at risk.

Automated tools can help. Once only within reach of the most well-funded, high-volume labs with specialized technicians on staff, automated library preparation is now accessible to nearly any NGS lab interested in streamlining its process. Automation has become more affordable and much simpler to operate. Complexity is no longer part of the picture—unless you want it to be.

Options today range from robotic liquid handlers that can be programmed to your needs, to microfluidics instruments that require minimal setup to prepare libraries for a wide range of applications.

In 5 steps, this guide will walk you through the range of automation tools that exist for next-generation sequencing library preparation, and help you find a solution that matches your application, throughput, and budget needs.



Step 1. Get Started: Identifying Your Needs

Let's begin the buying process by understanding your library preparation needs in the context of the next-generation sequencing work you'll be performing. Here are some of the key factors to consider.

Project and batch size. Calculate how many samples you expect to run per day, week, and month. Certain automation tools are better than others for high-throughput or low-throughput labs. There may be a minimum batch size requirement, as well.

Budget. Beyond the initial purchase price, factor in the ongoing cost of reagents and consumables, such as pipette tips, if required for the solution you're considering.

Time. The goal for most people is to spend as little time as possible managing and monitoring the library preparation process, freeing their day for other valuable tasks. An important associated factor is "walk-away" time, which is the ability to truly leave the lab without having to intervene at various points of the preparation process. By increasing walk-away time, it's possible to move beyond hands-on work to accomplish other important research activities.

Breadth of NGS applications performed. You'll want more flexibility if you anticipate that your lab's applications will change over time. For example, a lab running mostly DNA assays may consider whether it will be running RNA assays in the future. Depending on the level of change you anticipate, find out how readily automated systems will be able to adapt to these new needs.

Technical expertise. Every system has its own software and requires certain skills to assemble methods together that enable the application. Based on the level of complexity, determine whether you or someone else in your lab will be comfortable with programming that may be necessary. Alternatively, you may consider a load-and-go solution that doesn't require specialized training.

Availability of sample material. If you have access to only a limited quantity of sample material, you'll need a system that can accommodate a low input for library preparation.

Reproducibility. Consider your level of tolerance for error. Clinical labs, for example, tend to have an extremely low tolerance. Consider the costs associated with errors and what additional steps you have to take to account for them. Also, determine how you would like to track samples to ensure you're capturing all information.

Turnaround time. Calculate how much time you can afford to spend on library preparation. Some solutions will work faster than others.

After considering these factors, it's time to look at the product options and how they match up to your needs.

Step 2. Understand the Landscape: Your Library Preparation Options

Along with the growth in next-generation sequencing has come an expansion in the number and variety of library preparation solutions. While this variety is welcome and needed in the marketplace, it can add complexity to the decision-making process. To help you narrow your choices, here's a look at the product categories and methods that exist today, along with some of their key features.

Manual

Options	Benefits	Potential Challenges
<p>Capital Cost: Low.</p> <p>Number of Samples: Low.</p>	<ul style="list-style-type: none"> No major capital expenditure, as it does not require a dedicated system (although it does require the use of ancillary equipment and tools that labs may not already own). Is more flexible to incorporate changes to a protocol or method. 	<ul style="list-style-type: none"> Large quantity of consumables to purchase and manage. Requires high level of hands-on attention. Results are subject to variability, depending on who is running the protocol. Requires skilled technicians. More prone to errors than other methods.

Microfluidics

Options	Benefits	Potential Challenges
<p>Capital Cost: Low to high range.</p> <p>Number of Samples: Microfluidics instruments cater to different ranges of throughput, from low to high (depending on the application and platform). Multiple microfluidics instruments can work together to allow for higher-volume processing.</p>	<ul style="list-style-type: none"> Reduced hands-on time. High reproducibility, with reduced chance of human error. Some systems are dedicated to one type of application or have a narrow focus; others are more flexible and offer a broader set of applications. May enable lower sample input; favorable for situations where sample material is scarce. 	<ul style="list-style-type: none"> Normally requires use of assays supported by the equipment vendor. Ability to customize assays is more limited than with robotic liquid handling solutions.

Robotic liquid handlers

Options	Benefits	Potential Challenges
<p>Capital Cost: Mid-range to high, with some lower-range options.</p> <p>Number of Samples: Most are geared for high throughput. Some mid-range and lower-throughput options are available, but require more user intervention.</p>	<ul style="list-style-type: none"> Increases reproducibility with reduced hands-on time. Can be programmed for various lab needs, making it ideal for labs that require high flexibility. Allows researchers to adjust experimental conditions. Open robotic liquid handlers do not require reagents from specific vendors. 	<ul style="list-style-type: none"> Can use a large amount of consumables. Less capable systems may require substantial user intervention, reducing walk-away time. While being highly flexible, users do need to have programming expertise to develop or tweak assays. Ensuring that new programs work consistently and accurately demands time for validation and optimization. Changes to protocol may also require validation. The most highly automated walk-away options are expensive; can cost upwards of \$150,000 (USD).

No Limits: Rethinking Automated Capabilities

Automation has evolved to an extent that it's no longer safe to make assumptions that certain applications only work with certain types of automated library prep technologies. Take whole genome sequencing, for example. Microfluidics hasn't always been considered an option for whole-genome library prep, but today it is. For both microfluidics and robotics, sample kits today are simpler and more automation friendly, enabling researchers and clinicians to expand the boundaries of what's possible.

Step 3. Budget For Your Purchase: What Will It Really Cost?

Budgeting for your library preparation solution requires understanding your automation workflow and the time savings it will enable. Consider the initial capital expenditure as well as ongoing costs. Be sure to determine the cost of consumables that will be required to run your anticipated volume of samples. Here are tips to help you focus on the tools that will be the best fit for your budget.

Initial capital outlay. The upfront cost is an important consideration, but just looking at 1 price doesn't tell the whole story. The cost per sample is a more holistic approach to comparing systems.

Reagents. Evaluate the estimated cost of reagents required to perform library preparation on the system. Robots may require larger volumes of reagents to account for dead volume, which could increase the cost per sample.

Consumables. Consumables can vary across solutions. As with manual methods, robots usually require large numbers of disposable pipette tips, which can be expensive. Other consumables, especially plates, can also add up. Microfluidic solutions tend not to use tips, but require chips or cartridges.

Time and Labor. Hands-on time can range from more than 10 hours for a manual process to as low as 30 minutes for a load-and-go microfluidics system. If you're considering robotic liquid handling systems, find out how much technical skill is required in setting up new assays and performing needed interventions. For manual prep, you must have the appropriate staff, with the right skill sets, as well as substitutes in case someone is sick or on leave.

Service and Support. What are the expected maintenance expenses? Learn what type of maintenance will be required and how often. Depending on the system, maintenance tasks may require skilled engineers. Consider how much non-productive downtime you can afford while waiting for the instrument to be fixed. These are all factors to account for when negotiating a service contract.

Cost of Mistakes. In short, how easy is it to mess up? And what does an error mean to your lab, as well as those who are relying upon your data? As you evaluate options, look at the potential for mistakes and the ramifications. For example, with manual methods, your sequencing results could be unreliable if you pipette incorrectly. Also, what financial gains will reproducibility bring you? Whether you want to be published in a respected peer-reviewed journal or you are working with a clinic that depends on your data, this is a core issue to consider.

TIP: Explore Integration Software, Hardware, Workflow

It's generally a smart idea to choose a product that integrates with other steps in the sequencing and data analysis process.

- When evaluating robotic options, consider the costs and requirements of integrating with other lab equipment, such as a plate sealer, centrifuge, and microplate reader.
- With microfluidics, learn which tasks of the library prep workflow are included as part of normal operations.
- Pay attention to workflow and information management: Does the system you're considering integrate seamlessly with a Laboratory Information Management System (LIMS)? Does it provide an easy way to track your samples otherwise?

Step 4. Evaluate Vendor Support: It Really Matters

When investing in a next-generation sequencing system, understanding the level of support you'll receive from the vendor and its service team is a key component of your evaluation process. The same applies to automated library preparation technology. Here are some areas to consider, along with questions to ask of the vendor.

- 1. Customer support.** From training to troubleshooting, you'll want to ensure that the vendor will be responsive and knowledgeable. For example, learn whether the company offers in-house experts who can answer your questions promptly and help you get to the root of a problem. This is a key consideration, especially if you don't have an expert on hand in your lab.
- 2. Vendor's level of application expertise.** How broad is the vendor's menu of applications? Some automated solutions were developed for applications not related to NGS but have been adapted to meet this growing market area. If this is the case for a product you're considering, be sure that the vendor offers application experts who are familiar with the type of sequencing applications your lab performs.
- 3. Vendor's track record with emerging applications.** Does the company regularly release new assays to address emerging applications? If you must rely on your equipment vendor to supply assays, be sure that the company is responsive to new trends in sequencing and has a history of anticipating market needs. You don't want your sequencing progress to be held back because you don't have an assay to support it.
- 4. Workflow.** It's important for your automated library preparation system to fit into your larger NGS workflow. Explore how it will impact your daily work and meld with existing processes. Your chosen vendor should be experienced in adjusting and optimizing workflows to help you fully realize the potential of your new automated system.



Step 5. Make a Decision: The Checklist

There's a lot of information to grasp as you begin the buying journey—from anticipating your lab's needs and learning about the various product options to considering all the financial details. Use this guide to narrow your options for automating library preparation and ultimately choose which approach is best for you.

✔ I've identified my needs.

Understand your library preparation needs in context of the next-generation sequencing work you'll be performing:

- Project and batch size
- Budget
- Availability of time
- Breadth of NGS applications performed
- Technical expertise
- Availability of sample material for input
- Reproducibility
- Turnaround time

✔ I understand the market landscape.

To help you narrow your choices, here's a look at the product categories and methods that exist today, along with some of their key features.

- **Manual**—Low capital cost. Low throughput. Easy to change or modify protocol. Time consuming, prone to manual errors, requires hands-on attention. Large quantity of consumables to purchase and manage. Subject to variability depending on who is running the protocol. Requires skilled technicians.
- **Microfluidics**—Wide price range, from low to high, with many mid-range options. May enable lower throughput, depending on the platform. Reduced hands-on time. High reproducibility, reduced chance of human error. Lower sample input. Simpler protocol validation. Normally requires use of an assay supported by the equipment vendor.
- **Robotic liquid handlers**—Fully automated options are expensive. Most often used for high-throughput applications. Highly flexible, but often requires user intervention during the process, and programming expertise is needed to develop or tweak assays. Can be expensive and use a large number of consumables. Often requires user intervention at different parts of the process.

✔ I've budgeted for my purchase.

Budgeting for your library preparation solution requires understanding the various costs that will be required in the purchase and maintenance of the system, as well as the time savings it will enable for your lab.

- Initial capital outlay
- Reagents
- Consumables
- Time and labor
- Service and support
- Cost of mistakes
- Software, hardware, and sample tracking

✔ I've evaluated vendor support.

Understanding the level of support you'll receive from the vendor and its service team is a key component of your evaluation process.

- Is training available?
- What does the service contract include?
- What is the vendor's level of application expertise?
- Is the company on top of emerging applications? And does it regularly release new assays that address these applications?
- Does the vendor understand your full workflow and help you optimize it?

Know the Terms: Glossary

Consumables: Reagents, pipette tips, and other goods required for successful library preparation. Prices must be factored into ongoing costs and should be considered as part of the purchase process.

Hands-on time (also: HOT): A critical workflow element to consider; refers to how much manual labor or in-person attention is required in the lab to move a sample through the library preparation and next-generation sequencing procedure.

Data analysis: Software tools and computing methods that guide you from the original design of your experiment to interpreting data and generating actionable reports. For automated library preparation, consider complementary tools that will help manage samples, track success of the process, and share information with others.

Library preparation: A coordinated series of standard molecular biology reactions to prepare sample material for the next-generation sequencing workflow. Library preparation protocols can vary greatly according to application and throughput; can be performed manually or automatically.

Microfluidics: A technology that works with small volumes of fluids. For library preparation, this is a category of automated systems that can cater to different ranges of throughput, from low to high, depending on the application and platform. Provides high level of consistency and requires fewer steps and less hands-on time than manual methods.

Reagents: A substance or mixture for use in chemical analysis or other reactions. In next-generation sequencing, reagents are used to extract and enrich DNA and RNA samples, construct libraries, and quantify results, among other things.

Reproducibility: Consistent and reliable results when performing the same process multiple times, starting with the same sample and following the same protocol. Reproducibility is weighted heavily by peer-reviewed journals and is a priority for many clinical and research applications.

Robotic liquid handling: A robot that dispenses a selected quantity of reagent, samples, or other liquid to a designated container. Offers consistency and accuracy over manual methods; often used for high-volume samples. Flexible option for labs that lack predictability of applications to be performed.

Walk-away operation: The ability to walk away from an automated instrument while it performs its tasks. Different instruments allow for more walk-away time than others; this feature is important to consider when evaluating potential solutions for your lab.

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