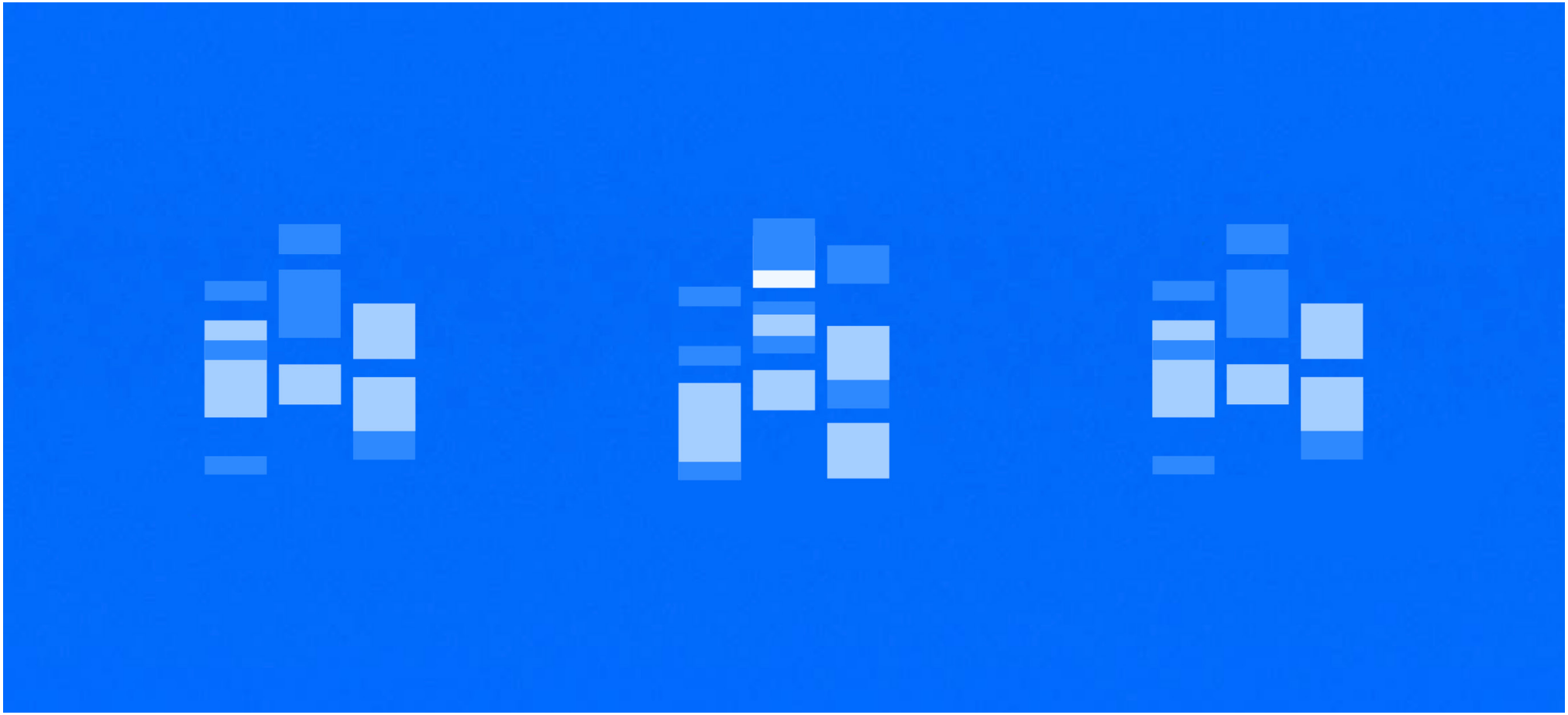
Automating NGS Library Preparation

Written by
Opentrons



INTRODUCTION

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CHAPTER 1

The Benefits of Automating NGS Library Preparation

CHAPTER 1

Improve Data Quality

- Accuracy & precision
- Reduced variability & errors
- Reproducibility & consistency

More Throughput in Less Time

Reduced Costs

Is automation worth it for your lab?

Valuable NGS data depends on quality library preparation; insightful sequencing relies on unbiased library coverage with good yields.

Unfortunately, library preparation is time consuming, labor intensive, and prone to errors. In the face of these challenges, quality library prep costs time and money, but scientists rarely have either in abundance. Thus, researchers find their ambitious goals constrained by a library-preparation bottleneck. Now, with over a decade of optimization and refinement, developments in laboratory automation promise to alleviate this constraint.

Automation has led to many benefits for NGS library preparation. This chapter will discuss three of the most important benefits: improved data quality, higher throughput, and reduced costs. Each factor will be discussed with an eye on aspects that might surprise those who are new to automation and an emphasis on aspects that impact research goals most.

IMPROVE DATA QUALITY

Accuracy and Precision

Accuracy and precision are critical in achieving desirable results at each step in the library preparation workflow. Across all tasks, reagents and samples must be aliquoted, combined, and transferred in precise amounts to drive sensitive chemistries and deliver quality results.

Most labs will likely need to pipette volumes down to 1 µl during NGS library preparation. At that volume, automated systems and skilled human technicians perform comparably, delivering actual pipetted volumes within 5% of the target, i.e., 0.95-1.05 µl, termed the coefficient of variation (CV). Human technicians and automated systems do differ in pipetting variability and error rate, as discussed below.

Reduced Variability & Errors

Automated systems minimize variability and eliminate errors, whereas technicians can struggle with these aspects when working with a complex protocol or a large volume of samples. Library

preparation protocols can entail myriad individual steps that are stretched out over an hours-long procedure, and given the size of most NGS experiments, pipetting tasks are repeated countless times over a full project (see chapter two for more discussion).

Inevitably, even skilled human technicians will suffer lapses in attention and commit pipetting errors. Often, the only solution is to redo entire procedures.

Robotic systems, however, can tirelessly and flawlessly execute pipetting. The right liquid is moved to the right well every time. Costly reagents and valuable time are saved by eliminating reruns (see more on cost savings below). Further, by eliminating errors automation delivers more dependable libraries and down- stream data.

Reproducibility & Consistency

Assays need to be reproducible and consistent, not only to ensure their outcomes are correct, but also to give researchers confidence in their results. Automation supports assay reproducibility and consistency better than manual processes.

One of the key factors that undermines assay reproducibility is variable pipetting. Automation eliminates variability in experimenter technique, because the robot performs each step in the same manner every time. Further, this supports better downstream analysis.

MORE THROUGHPUT IN LESS TIME

Aside from improving data quality, automation can increase a lab's throughput, producing more libraries faster and freeing up valuable hands-on time. Manual procedures take 3-4 hours each. In contrast, an automated system can complete a library in less than 3 hours, with less than 15 minutes of hands-on time, and the automated system can work off-hours. With faster turnaround times, automation can shorten experiments and deliver impactful data sooner. With higher-volume throughput, automation can facilitate more ambitious experiments and open new avenues for experimental design.

> **With less hands-on time, researchers can leverage their valuable skills by devoting more attention to experimental design and data analysis.**

REDUCED COSTS

Automated systems can require a significant initial investment, though some modern systems are much more affordable (see chapter 3 for tips about comparing automation options). But with gains in efficiency and performance, many labs will recoup their initial investment and in-fact enjoy savings in total costs.

Replacing tedious manual pipetting with efficient robotics cuts costs in many ways. By eliminating the need to conduct reruns following erroneous procedures, automation saves on wasted samples, reagents, and consumables. Further, by shortening turnaround times, automation saves the overhead costs of lengthy experiments. Despite the seemingly significant short-term investment and learning curve, the upshot of these improvements is net-savings for most laboratories.

The amount a lab might save with automation can be calculated.

Estimating your Expected Return on Investment (ROI)

A lab's ROI from automation can be calculated as the difference between their current costs with manual workflows and their potential costs with an automated system. These costs will depend on some lab-specific details, e.g., how expensive specific reagents are or how expensive hands-on time is. The biggest influence for ROI, though, will be the lab's throughput, as it can magnify both costs and savings.

To quantify costs, it helps to distill samples, reagents, consumables, labor, and machinery into a single metric, cost per sample (CPS). Labs can use their CPS as a baseline to extrapolate with their current and future throughput and compare against the costs of automation.

To calculate your CPS, add up the lab's assay expenses for a given period. Next, add in a measure of the labor hours spent on manual procedures. The metric, full-time equivalent (FTE), can be used to quantify such hours. FTE assigns an hourly wage to the work being performed. This can be the actual wage of a technician or a reasonable estimate. You can also quantify FTE in terms of hours rather than dollars, to focus on savings of time. Then, divide these costs by the number of samples run in that period. The quotient will be your CPS, either in terms of dollars or dollars and FTE hours per sample.

The next step towards calculating ROI is quantifying your CPS with automation. Simply add up the reagent and FTE costs for an equivalent number of samples using the new system. Then add in the costs associated with the automated system itself such as the purchasing price (see chapter 3 to ensure you are aware of sometimes-hidden costs). Divide this sum by the number of samples you intend to run, and you will arrive at a comparable CPS.

Automated systems with good ROI will offer affordable machines with minimal operating costs and deliver results that reduce costs as discussed above. The more samples you run, the more these net savings are magnified, largely by saving ever more hands-on time and reducing more erroneous assay waste.

Cost Per Sample: Manual vs. Automated

$$\text{COST PER SAMPLE} = \frac{\text{Lab assay expenses (e.g., reagents)} + \text{Labor hours spent on manual procedures (e.g., FTE)}}{\text{\# of samples run in a given time period}}$$

$$\text{COST PER SAMPLE (AUTOMATED)} = \frac{\text{Lab assay expenses (e.g., reagents)} + \text{Labor hours spent on manual procedures (e.g., FTE)} + \text{cost of automated system}}{\text{\# of samples run in a given time period}}$$

IS AUTOMATION WORTH IT FOR YOUR LAB?

Every lab has its own resources and goals. Many researchers rightfully wonder whether the benefits of automation will come to fruition in their own labs. Though recent advancements in automation have democratized their use and opened their benefits to most laboratory contexts, it is true that some labs might be better off sticking to manual procedures. Consider the following issues when evaluating automation for yourself.

Review these factors in the context of your lab goals and current resources to determine if your lab would benefit by moving to automation or if sticking with manual procedures makes more sense.

Factors that support sticking to manual procedures

- Minimal throughput needs, i.e., fewer than 10 samples per week
- Available laboratory resources (funding and labor)

Factors that support moving to automation

- Medium- to high-throughput sample processing
- High reproducibility required
- Need to reduce variability
- Need to protect staff from hazardous materials (in samples or reagents)
- Need to eliminate reruns
- Need to eliminate batch effects
- Can benefit from faster turnaround times
- Can benefit from larger experiments
- Can devote time to more productive tasks

CHAPTER 2

Thinking Through the Transition to Automation

CHAPTER 2

Evaluating Your Current Workflow

- Map your workflow
- Identify risky and/or bottlenecked manual steps
- Evaluate your throughput needs
- Evaluate your existing instruments
- Evaluate your sample needs

Understanding the Pressure Points and Optimization Solutions for Your Workflow

- Isolation and purification
- Quantification
- Mechanical and enzymatic fragmentation
- Enzymatic reactions: end repair, adenylation, ligation, tagmentation
- Amplification
- Size selection/clean-up
- Normalization

Summary of Pressure Points and Optimization Solutions

If you're like others, it may be your first time moving from a manual process to automation, given that the costs and technology have only recently become accessible.

Luckily, over the years many researchers and laboratories have gone through this transition, and common principles have come to light regarding how to do so successfully. The discussion below will walk through the process and describe the key points to keep in mind. Further help with transition from manual to automated protocols is often provided by automation companies.

EVALUATING YOUR CURRENT WORKFLOW

The available automation systems on the market offer a range of features with various strengths and weaknesses (see chapter 3 for tips about comparing these systems). So, before you know which systems are the best for you, it will help to identify your needs. By following these steps, you will arrive at a clear picture of your needs and will be well prepared to compare available systems.

Map your Workflow

First, start with these basic processes: quantification, fragmentation, end repair, adenylation, adapter ligation, size selection, clean-up, and amplification. Then list your current workflow's techniques for each process.

Identify Risky and/or Bottlenecked Manual Steps

Risky steps that bottleneck your throughput will be priorities for automation and will inform the critical needs of your automation plan; improved performance and speed will have the most impact on these steps. Procedures that require lengthy pipetting and/or several changes in reagent mix are particularly prone to human error. They also tend to take the most time. Enzymatic steps such as end repair, adenylation, and adapter ligation fit this bill.

Evaluate Your Throughput Needs

As you consider the bottlenecks in your workflow, it will be a good time to think about your throughput goals, and how your workflow matches up, to get clear about how problematic these bottlenecks are. Ask whether you are generating as many libraries as quickly as you need them. In this light, also

Map Your Workflow

QUANTIFICATION
Spectrofluorometric analysis or bioanalyzer

FRAGMENTATION
Sonication or enzymatic

END REPAIR
Enzymatic mix

ADENYLATION (A-TAILING)
Enzymatic mix

ADAPTER LIGATION
Enzymatic mix

SIZE SELECTION
Magnetic beads

CLEAN-UP
Magnetic beads

AMPLIFICATION
PCR

Common risky and slow steps

This is an example of a common NGS workflow. Actual steps may vary depending on the assay.

look ahead to your future goals. As academic and industry research progress, genomic scientists find value in ever more ambitious experiments. If you see value in scaling up your NGS workflows in the future, the bottlenecks will become even more impactful.

Evaluate Your Existing Instruments

List the instruments that you currently rely on in your workflow. Then consider how they are contributing to or impeding your research goals. Are they achieving adequate precision? Do they run enough samples to support your throughput needs? Is instrument maintenance a challenge for the lab, costing significant time and/or money? For machines that rely on software or digital protocols, are those tools and interfaces working well; are they saving you hands-on time and delivering consistent data quality? Note any issues or sore points that have come up in your lab operations. They might be resolved by automation, and they might become important in choosing between automation systems.

Evaluate Your Sample Needs

Various sample types require specific reagents provided in appropriate kits while automated systems themselves can be better or worse suited for your kits and sample types. So, you'll want to have your sample needs in mind when selecting between the various robots.

Key aspects of your samples to consider are differences in their physical, chemical and molecular properties, viscosity, concentration, hazards, and special handling requirements to name a few.

Each of these factors can influence the sort of robot you need and are key requirements in your transition to automation. If you work with highly viscous samples, you'll want to ensure your robot is calibrated and programmed to handle the liquids' viscous nature appropriately. Similarly, if your samples are especially diluted or concentrated, you might need to adjust your workflow, and you'll want a system that can accommodate this. If you work with hazardous samples, e.g. blood with pathogens, they are particularly suitable for automated workflow, as hands-on time increases health risk. Some sample types can also benefit from specific automation solutions. For example, high-volume work with blood samples can benefit from capping/de-capping systems.

UNDERSTANDING THE PRESSURE POINTS AND OPTIMIZATION SOLUTIONS FOR YOUR WORKFLOW

Each procedure in the library preparation workflow has its own set of challenges. The specific tools and desired endpoint of each step create pressure points that labs will experience when trying to generate optimal results. Automation offers an effective means of optimizing library preparation, but care must be taken to ensure that the solutions are aligned with the lab's pressure points and goals. Automation companies may have Customer Success Teams and other experts who can help you find the right solutions for your specific challenges and goals.

YOUR SAMPLE

As discussed above, various aspects of your sample will influence your workflow - the reagents you use, the machines you need, etc.. Your sample also gives rise to particular pressure points which you'll want to keep in mind, and which might be amenable to automated solutions. Sample types and their associated pressure points can vary widely between labs, but you are likely very familiar with your own samples. Make note of the pressure points you've found and keep them in mind as you consider the workflow.

This section will help prepare you for the transition to automation by describing each step in the library prep workflow with an eye on the pressure points and solutions that might apply to a particular lab.

Isolation and Purification

Prior to library preparation, most labs isolate and purify their DNA from some sort of raw sample. In fact, isolation and purification is its own workflow that is upstream of many molecular biology applications and has its own set of steps with their own pressure points. Luckily, some modern automation systems are flexible enough to perform both workflows (and more). See chapter 3 for discussion of automated system flexibility.

Quantification

Since library concentrations can be very different, library prep requires quantification before sequencing to ensure that each pooled library is sequenced to the desired depth. This supports optimal sequencing outcomes. Quantification, normalization, and pooling are critical to NGS results because inaccurate molar concentration can lead to over-loading, which will degrade resolution and data quality, or under-loading which will reduce data output. These steps should be optimized for throughput for your lab, as some common methods (e.g., PCR) can be quite time consuming and are at risk for assay variability.

Biased sequencing reads can be corrected by careful optimization of a quantification kit within the specific parameters of the lab's workflow, i.e., sample type, sample volume, starting concentration, etc.. Kit and/or machine vendors should support this process, though they do vary in this regard (see chapter 3 for more discussion).

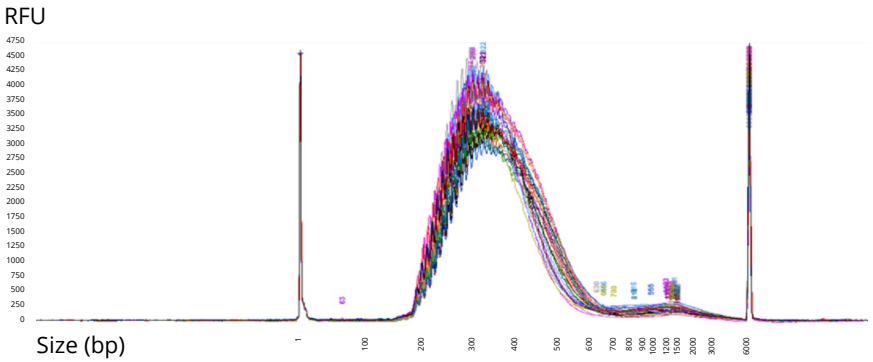
The simplest and often most effective way to improve quantification accuracy is by using adequate replication, running replicate samples while monitoring to ensure consistency between them **(figure 1)**. Depending on the kits and systems used, this can add time to the workflow. But inaccurate reads are much more impactful on throughput, and the added time for further replicates can be overcome by other methods.

Automation is far and away the strongest means of improving throughput. As discussed in the previous chapter, robotic systems need less hands-on time and can operate at a faster rate and over longer periods of time than manual procedures.

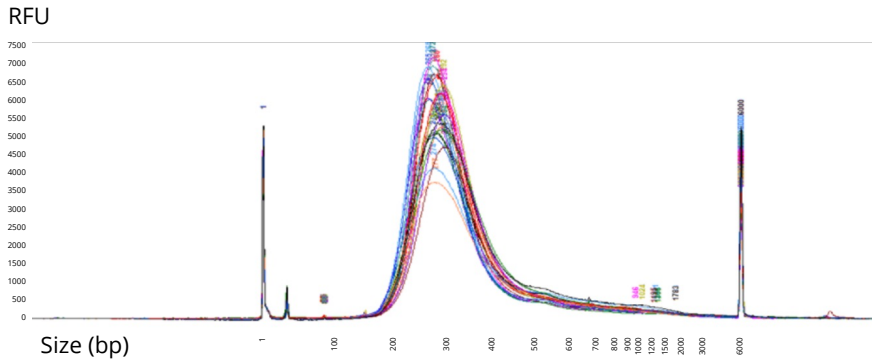
PRESSURE POINTS	OPTIMIZATION SOLUTIONS
Read Bias	Validated kit for workflow
Read Accuracy	Adequate replication
Throughput	Automation

Figure 1. DNA Fragment Sizes

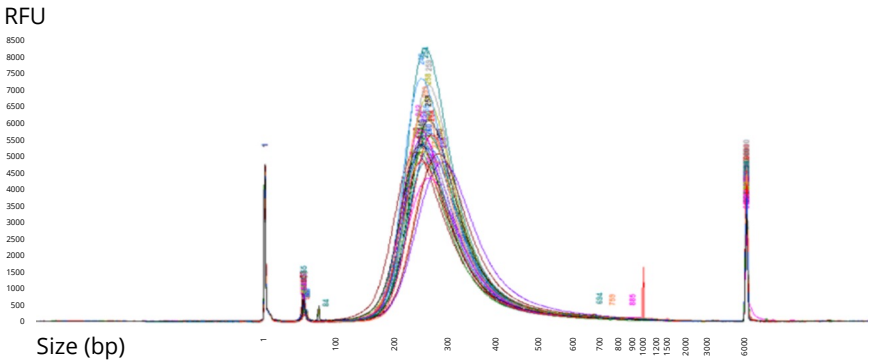
Reliable uniformity of DNA prep libraries prepared on the OT-2 are shown. An Agilent 5300 Fragment Analyzer was used to calculate fragment sizes of DNA prep libraries using kits from three different manufacturers (Illumina, Roche, and NEB).



Illumina



Roche



NEB

Mechanical and Enzymatic Fragmentation
Optimal fragmentation for library preparation creates fragments with the following features:

- Randomized lengths
- Fragments that are within the target range
- A range of fragment lengths that is not overly broad

Randomization is important to ensure full, unbiased sample coverage, while the fragment range influences clustering and is determined by the downstream sequencing platform. Whether at long or short lengths, an overly broad range can lead to fragments falling outside of the desired range and result in biased coverage in downstream sequencing.

Fragmentation methods differ in the fragment sizes they can deliver. Short fragment lengths, in the range of 5 bp to 1 kb, can be achieved with acoustic and sonication methods, while for longer fragments, hydrodynamic fragmentation might be necessary.

A procedure's control over fragmentation range is often at odds with three other considerations: required input, required time, and upfront costs. Sonication methods tend to be slow, acoustic methods tend to cost more, and hydrodynamic methods tend to require more input.

Enzymatic fragmentation, as an alternative to mechanical methods, requires little starting input, has low costs, and can achieve high throughput, though it can suffer from sequencing bias and contamination.

Sonication is generally recommended as a good over-all fragmentation method, though enzymatic fragmentation performs well in many circumstances. Balancing these features to deliver the best quality fragmentation will depend on the details of a lab's workflow. Thus, optimization within lab is necessary.

Various automation solutions exist for each of the fragmentation methods; separate machines perform the different forms of mechanical fragmentation and enzymatic fragmentation can be handled by the same automation as other enzymatic reactions in the workflow (see below for more discussion).

PRESSURE POINTS	OPTIMIZATION SOLUTIONS
Fragmentation Range	Hydrodynamic and enzymatic fragmentation
Amount of Required Input	Sonication, Enzymatic fragmentation
Throughput	Automation, hydrodynamic and enzymatic fragmentation
Costs	Automation, sonication, acoustic and enzymatic fragmentation

Enzymatic Reactions: End Repair, Adenylation, Ligation, Tagmentation

Enzymatic reactions contribute to several different steps within the library preparation workflow. Though the reagents and end-products for each of these steps differ, their core procedure is the same – controlling temperature, i.e., thermocycling or incubation. Good enzymatic results will require good reagents that are optimized within the lab. But optimizing the temperature control procedures themselves is relatively straightforward.

Incubation and thermocycling devices generally set, hold, and transition through required temperatures with precision and uniformity. The main machine feature that can be improved is sample capacity to increase throughput. Likewise, scalability can be important as lab needs change. Some devices flexibly accept a range of plate blocks supporting future throughput increases, while others are more rigid and costly to upgrade.

To a greater degree, pressure points for enzymatic procedures will center around user intervention rather than the device. Pipetting errors, contamination, and variability between users can cause significant issues.

Automation is the best way to address these issues and increase throughput. Robotic pipetting systems can work with thermocyclers as well as static incubators to significantly reduce necessary hands-on time and, as described in chapter 1, robotic systems excel at these lengthy repetitive procedures.

PRESSURE POINTS	OPTIMIZATION SOLUTIONS
Throughput	Automation
Scalability	Flexible device
Errors and Variability	Automation

Amplification

Many library preparation workflows rely on PCR amplification in a thermocycler to both increase the concentration of genomic sample and to attach sequencing adapters. Indeed, adapter ligation during PCR is important for quality sequencing results.

PCR amplification suffers two pressure points: bias and yield. Bias results from inconsistent interaction between PCR technologies and extreme base composition of substrate nucleotides, meaning that some fragments will be amplified more than others. Biased PCR leads to uneven coverage in sequencing data, amounting to complete absence of coverage for some regions of the genome. Yield is important for all steps in library prep but becomes especially salient in optimizing amplification, as a tension exists between the solution to address bias and the need for greater yield.

Bias can be reduced by limiting the number of PCR cycles, but that also lowers output. Bias is also addressed by advancements to PCR and quality kits. Ultimately, the right balance between bias and yield will rely on calibrating with a lab's specific parameters.

PRESSURE POINTS	OPTIMIZATION SOLUTIONS
Coverage Bias	Quality kits, calibration
Yield	Quality kits, calibration

RNA Amplification

Here is an example of RNA amplification read from the Opentrons OT-2 system using human genomic input material.

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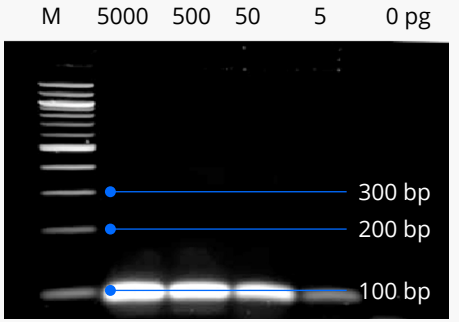


Size Selection/Clean-up

Size selection and clean-up processes eliminate contaminants that can disrupt sequencing. These contaminants include fragment lengths outside of the sequencing platform's range, as well as remnant oligonucleotides and reagents from earlier procedures.

For this step, magnetic beads are a popular tool, while spin columns and gels are other options with strengths of their own. Bead-based protocols are fast and easy to automate. They excel in cleanup performance and flexibility, though they deliver a relatively broad range of size fragments. Spin columns are relatively simple to use and are supported by some kits, but they are prone to clogging when highly concentrated samples are used. Gels are another straightforward technique and are well known by molecular biologists. They can be used when DNA fragment size is not known and for fine distinctions between the target nucleotides and contaminants (e.g., between miRNA and dimers), but gels take much more hands-on time.

Four core pressure points challenge this step: yield, consistency, specificity, and throughput. The various methods each involve some amount of transferring sample between treatments which inevitably involves some amount of loss. Likewise, the chemistries used have varying performance regarding DNA capture, and separation of the target nucleotides impacts yield. Consistency in selection and clean-up depends on pipette performance and is made particularly difficult by viscous samples and reagents. Finally, for manual procedures in particular, throughput lags.

Yield can be improved with high-performing kits and reagents and by minimizing loss to superfluous liquid transfer. Consistency likewise depends on quality reagents but can be improved with careful pipetting. Throughput can be improved significantly by using magnetic methods due to their minimization of hands-on processing.

Though all three discussed procedures can be automated, gel automation requires specialized machinery that can be expensive, and column automation still requires hands-on intervention. Magnetic bead-based clean-up is highly amenable to automation, including full end-to-end automation of the process with relatively low-cost robots.

PRESSURE POINTS	OPTIMIZATION SOLUTIONS
Yield	Quality kits, minimizing liquid transfer steps
Consistency	Quality reagents, improved pipetting, automation
Throughput	Bead-based methods, automation

Normalization

Many workflows use normalization, i.e., equalizing the nucleic acid concentration of each library prior to pooling and sequencing. Concentration influences read depth; the greater the concentration the deeper the reads. Low concentration and corresponding low read depth are particularly problematic, as they can reduce data quality. So normalization helps ensure adequate and consistent read depths are achieved for all libraries.

The process and pressure points for normalization will be like those mentioned for quantification above - quantification is the critical step for accurate normalization. However, some unique issues arise when quantifying for normalization, and a unique normalization solution can obviate quantification all together.

Three common quantification methods are used for normalization:

- qPCR
- Fluorometry
- Spectrophotometry

Among these methods, qPCR excels at specifically quantifying adapter-ligated strands, those that are amenable to sequencing, with high sensitivity and accuracy. But qPCR can be time-consuming and does not provide fragment size information.

Fluorometry is also relatively sensitive and accurate and is faster than qPCR. But fluorometry cannot distinguish between ligated and non-ligated molecules.

Spectrophotometry is the fastest and least expensive of these techniques but is also the least accurate, least sensitive, and cannot distinguish ligated and non-ligated molecules either.

A fourth option, magnetic bead-based normalization, can accurately and precisely equalize the volume of adapter-ligated molecules without the need of longer quantification steps. Beads are applied to the library and bind a quantity of molecules proportional to the quantity of beads. This method achieves accurate normalization with a relatively fast procedure, though beads can waste genomic material if concentration far exceeds their binding capacity.

Automation works best with qPCR and bead-based procedures, as these can be performed with the least manual interventions. Automated qPCR can resolve qPCR's demand for time, though automated bead-based normalization will offer the shortest turn-around-time.

PRESSURE POINTS	OPTIMIZATION SOLUTIONS
Accuracy	qPCR, magnetic beads
Sensitivity	qPCR, fluorometry, magnetic beads
Speed	Spectrophotometry, magnetic beads
Sample Conservation	qPCR, fluorometry, spectrophotometry

Concentration Normalization

The table below is a comparison of pre- and post-normalized DNA input. Input material (Lambda and Human Genomic) was normalized to 1 ng/µL, 2 ng/µL and 6 ng/µL. The normalized concentration values are within range of target concentration with CV's under 10%. Normalization is important to get accurate and consistent data quality in next-generation sequencing.

		Target = 6 ng/µl	Target = 1 ng/µl		Target = 2 ng/µl
	INPUT	NORMALIZED	NORMALIZED	INPUT	NORMALIZED
	8.14	5.67	1.17	7.79	1.8
	9.94	6.06	0.98	8.41	2.15
	16.8	5.88	0.91	9.69	2.02
	11.1	5.86	1.10	9.57	2.14
	7.28	5.61	1.14	8.71	1.91
	10.3	5.58	1.21	10.6	2.08
	9.11	5.7	1.09	9.3	2.16
	7.99	6.09	1.16	11.1	1.98
AVG	10.08	5.81	1.10	9.40	2.03
CV	29.8%	3.4%	9.4%	11.73%	6.34%

SUMMARY OF PRESSURE POINTS AND OPTIMIZATION SOLUTIONS

Several common principles stand out when considering how to optimize library preparation across the various workflow steps. Unsurprisingly, throughput, yield, performance, and cost are common pressure points across the workflow, though, as discussed above, each step has its particular performance needs.

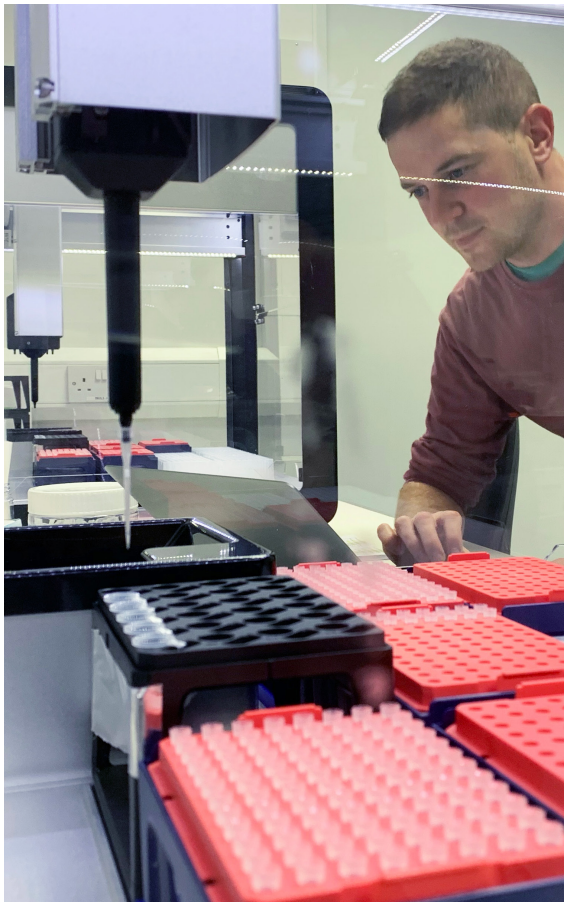
Common optimization solutions are also apparent. Firstly, automation presents an opportunity to address several challenges. By reducing errors, simplifying procedures, and increasing throughput, automation improves performance at each step, and these solutions can come at reduced costs.

Another common theme for optimization is well validated, quality kits and reagents. All processes involving enzymatic reactions rely on sensitive chemistries and sophisticated technologies where good performance relies on good materials and calibration with a lab's specific parameters (e.g., sample type, sample concentration, etc.).

Finally, two key technologies, enzymatic fragmentation and bead-based selection/clean-up, stand out as both optimizing several features of their respective procedures, but also fitting well with automation. Both technologies are flexible and easy to implement. For some specific applications, these techniques might not be appropriate, in which case selecting flexible automation solutions will be important for creating an optimal workflow.

Common Workflow Pressure Points

- Throughput
- Yield
- Performance
- Cost



CHAPTER 3

How to Choose the Right Automation Platform for You

CHAPTER 3

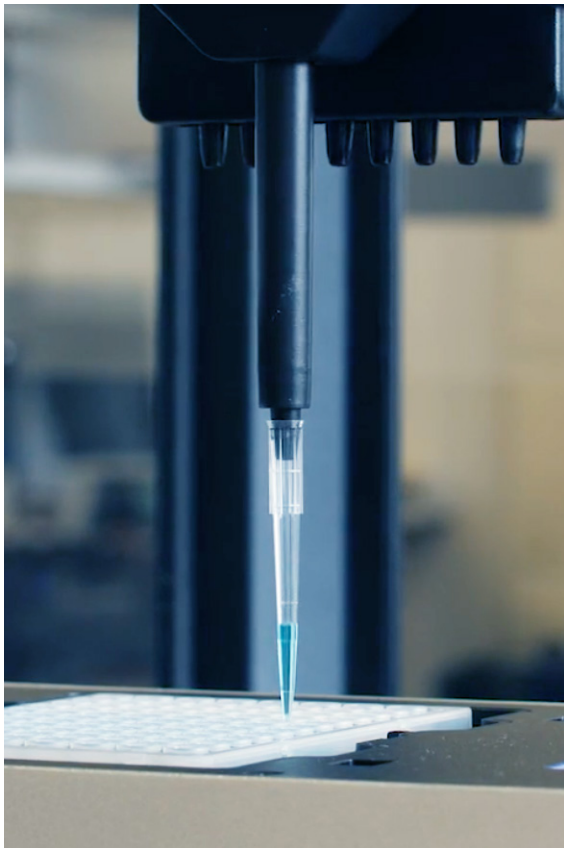
Which Robots Address Your Workflow Needs and Pressure Points?

Appreciating the Full Cost of Automated Systems

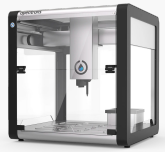
- Set-up costs
- Transition costs
- Operating costs

A range of vendors and their various robotic systems are available to automate library preparation.

Such a wide range of options exists that prices can range from less than $50,000 to greater than $200,000. Choosing among them can be difficult, especially as many important features about the systems, their practical use, and their true costs are not obvious. The discussion below will focus on these issues and try to make it easier to find the ideal system. See our Guide to Automation eBook for further discussion.



Liquid Handler



Thermocycler

Temperature Module

Magnetic Module

THE CORE MACHINES: WHICH ROBOTS ADDRESS YOUR WORKFLOW NEEDS AND PRESSURE POINTS?

Liquid Handler
The workhorse of automated systems for use in NGS library prep along with most other benchwork procedures in modern laboratories, a liquid handler robot will address the need for improved performance and throughput. Good liquid handlers will offer the flexibility to support a lab's workflow and provide the ability to scale with the lab's needs.

Thermocycler
Robotic thermocycler units are designed to fit with automated workflows.

Temperature Module
Used to support varying temperature requirements of reagents, enzymes and other fluids used in an NGS library prep assay.

Magnetic Module
Key module to deliver size selection and bead-based processing. Allowing the flexibility, ease-of-use, and throughput that leads to optimal results.

APPRECIATING THE FULL COST OF AUTOMATED SYSTEMS

Once you know which machines to consider, you can turn to selecting between the various vendors. Often the first aspect researchers consider when selecting vendors is cost.

Ambitious research goals and the desire to improve data quality are constrained by budget concerns. Unfortunately, cost-benefit analysis for automated systems can be complicated. Beyond the price of the equipment, there are several less-transparent costs, ranging from the initial purchase to day-to-day operations. Researchers can make more confident purchasing decisions by thinking through the following factors and understanding the full cost of a system along with the benefits of automating their NGS library preparation.

Set-up Costs
The full price to purchase an automated system and get it running in the lab includes the installation fee as well as the ticket price. While installation fees are often described as an optional service, many machines are large and use proprietary technologies

that necessitate on-site expertise for set-up. Aside from financial cost, delivery and installation can take time, delaying research progress. Unfortunately, some users are surprised by long lead times between placing their order and delivery of their machine. Finally, space is an oft-overlooked cost for most wet labs. Automated systems from large multi-machine units to simple benchtop stations take up valuable lab space and may not fit in your lab.

Transition Costs

After delivery and installation, labs need to adapt their manual protocols to their new devices. This transition will involve changes to workflow and creating or adjusting the new machine's software protocol. Such changes will require investments in time and training. How much investment is necessary will depend on how easy the system is to operate, what expertise lab members already have with the system or its protocol development methods (including coding), and what support resources the vendor supplies. Some machines have pre-programmed protocols that only their engineers can adjust, some have adjustable software that requires extensive training, while others have flexible open-source software with simple graphic interfaces. Modern systems go further by integrating and promoting online communities, like GitHub, where users can share protocols. In these communities, researchers can find pre-validated and pre-optimized protocols that they can trust.

Operating Costs

Like any lab device, any NGS library prep robot will require some amount of maintenance. But systems differ in how complicated they are to service. Vendor support services can range from expensive contracts for in-person support, to mid-tier contracts with a la carte services, to free web-based information hubs and remote technical support. The level of support and associated costs will depend on the system. The less user-friendly the device is, the harder issues can be to resolve, and the more time maintenance will require. For some systems, expensive service contracts and lengthy downtime are a necessary part of the true operating cost, while other systems are simple enough that free support services can keep them running optimally.

Along with maintenance, a new automated system might require new reagents, meaning expensive new purchases, and lost time to adjusting workflows and protocols. Some systems require specific techniques and corresponding reagents while other devices are more flexible. For example, if a lab's manual protocol uses magnetic beads for enrichment, a new system might require them to switch to spin columns. In contrast, a flexible system could be adapted to utilize magnetic beads, avoiding the purchase of new columns and the lost time to incorporate them into the lab's procedures. Further, some systems require the use of proprietary reagents, locking users into ongoing purchases from expensive vendors. Restrictions on reagent use also make labs prone to supply chain issues. If a lab's system must use a specific reagent and that reagent suffers supply shortages, the lab is stuck paying increasing costs and waiting for slowed delivery. Conversely, flexible systems allow users to pick from a range of reagents, selecting the most economical products that meet their needs.

Summary

Automation

- Improve Library Quality
- Increase Throughput
- Reduce Costs

Automation can improve library quality and increase throughput while reducing costs. Robotic systems can be incorporated into each step of the library preparation workflow as is appropriate for a laboratory's needs, or the entire end-to-end process can be automated with a comprehensive workstation. Performance at each of these steps will depend on alignment between a chosen system and the pressure points and specific needs that a lab faces. These points should be considered, along with the full costs of the available automated systems on the market. Though some costs are less transparent and often overlooked, they have come to light over the years the industry has served laboratories.

CONCLUSION

Next Steps in Automating Your NGS Library Prep

Hopefully, this e-book has provided helpful information and clarified the issues surrounding NGS library preparation automation. More information is available to address remaining questions. Follow the links below for more helpful information about automation for research laboratories:

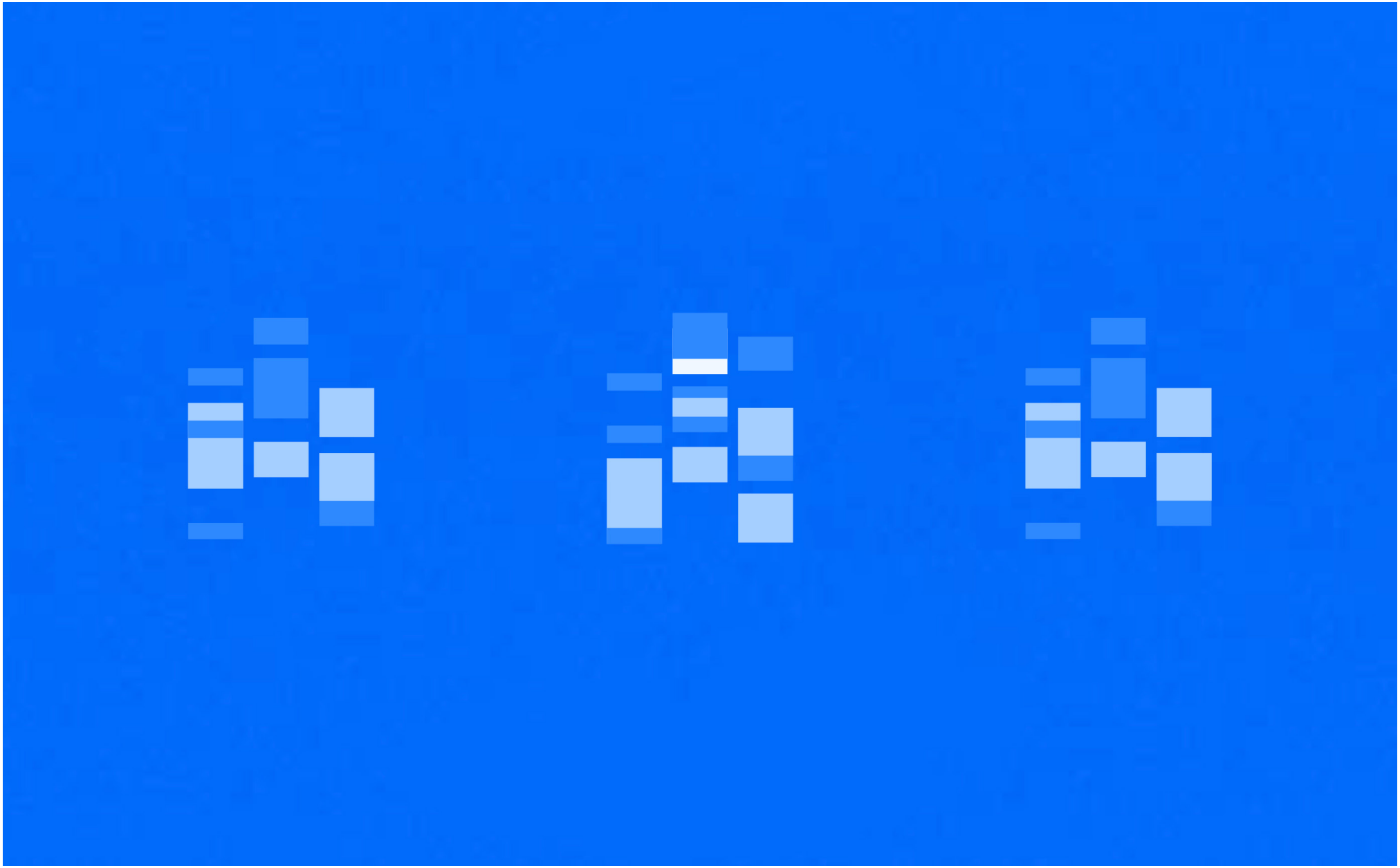
BLOG **NGS Automation**

BLOG **Assessing Good Quality DNA**

BLOG **Other E-Books**

BLOG **Opentrons User Interview: YouSeq's Tom Huckvale**

PDF **Viscous Liquid Handling**



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