Automating PCR

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INTRODUCTION

Table of Contents

CONTENT

CHAPTER 1

The Benefits of Automating PCR

CHAPTER 1

Improve Data Quality

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

More Throughput with Less Hands-On Time

Reduced Costs

Is automation worth it for your lab?

PCR is a molecular biology workhorse that is used for detecting and quantifying specific genes and is an important component of downstream applications including genomics and transcriptomics.

Unfortunately, PCR is time-consuming, labor-intensive, and prone to errors. In the face of these challenges, quality PCR costs time and money, but scientists rarely have either in abundance. Thus, researchers find their ambitious goals constrained by a PCR 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 PCR. 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

PCR consists of two workflow steps: 1) Reaction setup and, 2) thermocycling. Accuracy and precision are critical for plate loading and impact final results. Reagents and samples must be aliquoted, combined, and transferred in precise amounts to drive sensitive chemistries and deliver quality results. Thus, moving exactly the correct volume of sample and reagents is a critical performance factor that strongly influences product quality.

Most labs are likely to pipette volumes down to a minimum of 1 µl when performing PCR. At that volume, most automated systems and skilled human technicians perform comparably, delivering actual pipetted volumes within 5% of the target, i.e., 0.95–1.05 µl when pipetting 1 µl.

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.

PCR protocols can entail myriad individual steps that are stretched out over a sometimes hours-long procedure, and given the size of most PCR experiments, pipetting tasks are repeated countless times over a full project (see chapter three 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 accurate downstream 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 WITH LESS HANDS-ON TIME

Aside from improving data quality, automation can increase a lab's throughput, running more PCR reactions faster and freeing up valuable hands-on time. The length of PCR applications can vary considerably. For a typical procedure, PCR takes 1–2 hours, including setup and runtime. An automated system can complete PCR in a similar amount of time, but with less than 15 minutes of hands-on time, and of course, the automated system can work nights and weekends.

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

Manual PCR

TOTAL TIME
1-2 hours

HANDS-ON TIME
30 mins, or more for large numbers of samples

Automated PCR

TOTAL TIME
1-2 hours

HANDS-ON TIME
<15 mins

REDUCED COSTS

Automated systems can require a significant initial investment, though some modern systems are much more affordable. 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 4 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

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

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

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 optimizing with automation

- Medium- to high-throughput sample processing
- High reproducibility required
- 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

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 the 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 shop among available systems.

Map your Workflow

List the machines, reagents, and consumables you use throughout the PCR workflow. Also, consider if you are using, or will need to use quantitative, multiplex, or nested PCR. If so, list each step for these added procedures.

Evaluate your Throughput Needs

As you consider your workflow, it will be a good time to think about your throughput goals, and how your workflow matches up. Ask whether you are generating as much amplified DNA, as quickly as you need it. In this light, also look ahead to your future goals. As academic and industry research progress, molecular biologists find value in ever more ambitious experiments. If you see value in scaling up your PCR workflows in the future, any bottlenecks will become even more impactful.

Evaluate Reagent Needs

The reagents you use can impact compatibility with some automation systems, or if the reagents are especially viscous for example, they might raise pain points that an automated system must overcome. Which reagents you need will depend on several factors. If your sample is high in PCR inhibitor concentration, you might need reagents that are resistant to inhibition. If you have a low template quantity, you might need highly sensitive reagents. If your target has a complex structure, you might need to increase annealing temperature or add reagents to destabilize secondary structures. So, keep your reagent needs in mind as you evaluate automation.

UNDERSTANDING THE PRESSURE POINTS AND OPTIMIZATION SOLUTIONS FOR YOUR WORKFLOW

More subtasks might be included depending on which PCR application is used. In any case, each task has its own set of challenges. The reagents and processes of each step create pressure points that labs will experience when trying to generate optimal results. Automation offers an effective means of optimizing PCR, but care must be taken to ensure that the solutions are aligned with the lab's pressure points and goals. Many labs go further with customer success and support teams to pass on best practices.

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 pressure points that you'll want to keep in mind and 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 PCR workflow with an eye on the pressure points and solutions that might apply to a particular lab.

The simple PCR workflow breaks down into several subtasks:

PLATE LOADING

Add primers, enzyme, and other reagents to a master mix; load template; load master mix

THERMOCYCLING

- Denaturing
- Annealing
- Extension

Plate Loading

Plate loading is a series of pipetting and mixing steps and thus represents a source of several pain points. The goal is to produce highly accurate mixes of the appropriate ingredients while eliminating contamination. Such results require high precision, accuracy, and reliability. Further, lengthy pipetting creates a challenge for throughput goals.

Precision, accuracy, and reliability goals can be met by highly trained technicians who have the time and attention to dedicate to the task.

Likewise, careful set up and clear lab protocols can create an environment where good performance is made easier. Sophisticated pipettes can improve performance. And automated systems can achieve impeccable results.

Further concerns come from the templates and reagents used in this step. Plate layout can be an issue; many reagents arrive in plate formats that don't match the PCR plate. So, plates must be reformatted, often requiring single-channel pipetting.

Reagents themselves can create issues; some must be maintained at specific temperatures, and some require challenging pipetting to overcome viscosity. Temperature requirements can be resolved by working on ice or incorporating temperature modules into automation and working quickly helps minimize exposure to room temperatures. Viscosity must be overcome with careful pipetting, either with specialized training for manual procedures or flexible systems for automation and low retention consumables.

PRESSURE POINTS	OPTIMIZATION SOLUTIONS
Precision, Accuracy, and Reliability	Extensive training, well-developed protocols, advanced pipettes, automation
Plate Layouts	Plate reformatting, single-channel pipettes
Temperature Requirements	Ice, temperature modules, quick procedures
Viscous Reagents	Specialized training, flexible automated systems, specialized consumables

Thermocycling
This step is largely performed by a machine, but nonetheless, researchers run into issues trying to consistently achieve good results. As discussed above, several specific factors from your application and workflow determine the optimal thermocycling parameters. However, across applications, the goal is to achieve highly accurate, highly precise replication at high yield. Achieving these results will require piloting variations in the parameters and performing quality control to observe the output and inform adjustments.

Further, specific issues can arise in the use of a thermocycler. Submitting the liquid PCR ingredients to cyclic temperature changes raises the potential for evaporation. And inconsistent heat distribution can cause plate-position artifacts in data as individual plate wells vary in heat exposure. Both of these effects reduce data quality and slow throughput as they consume time to fix.

Evaporation can be prevented with the selection of effective sealing systems. And plate-position artifacts can be prevented with the selection of thermocyclers that exhibit uniform heating. Both issues can be monitored and adjusted with appropriate quality control measures.

Specific Liquid Handling Needs
The range of liquid volumes you work with can give rise to specific challenges. And this can impact each step as volumes change. Extremely low volumes raise specialized needs for pipetting accuracy. Extremely high volumes can raise specialized needs for labware and make throughput a challenge.

PRESSURE POINTS	OPTIMIZATION SOLUTIONS
High Accuracy, Precision, and Yield	Piloting within lab context and adjusting parameters, effective QC
Plate-position Artifacts	Uniform heating, QC
Evaporation	Effective sealing, QC

SUMMARY OF PRESSURE POINTS AND OPTIMIZATION SOLUTIONS

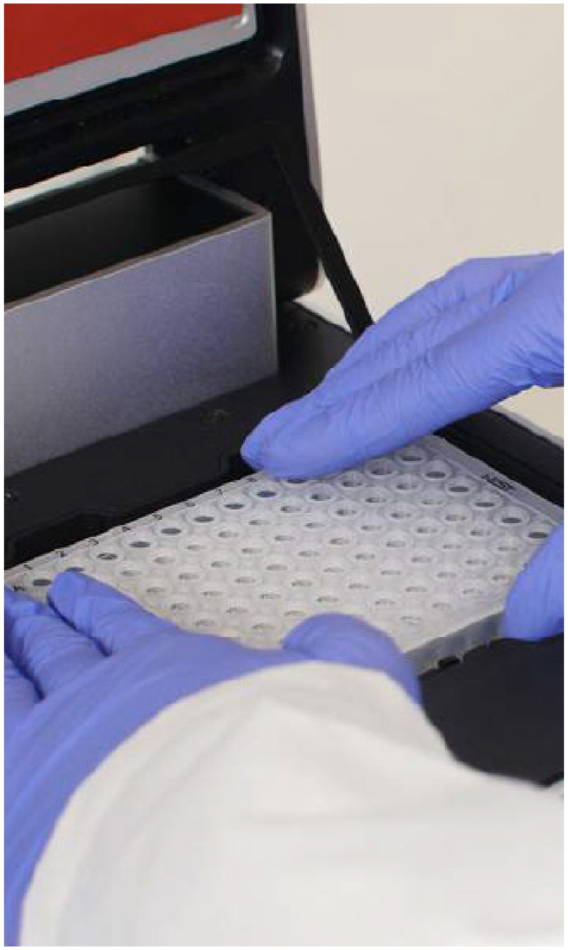
Several common principles stand out when considering how to optimize PCR across the various issues and workflow steps. Unsurprisingly, throughput, yield, and performance, are common pressure points; though, as discussed above, each step has its particular performance needs.

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 quality control. Many PCR parameters depend on interrelated needs among the specific template, reagents, and machines a laboratory uses. Thus, piloting and adjusting are paramount to good results. But such adjustments necessitate effective quality control measures. Automation helps here as well, as it increases reproducibility, to deliver clear results when changes are made.

Common Workflow Pressure Points

- Throughput
- Yield
- Performance

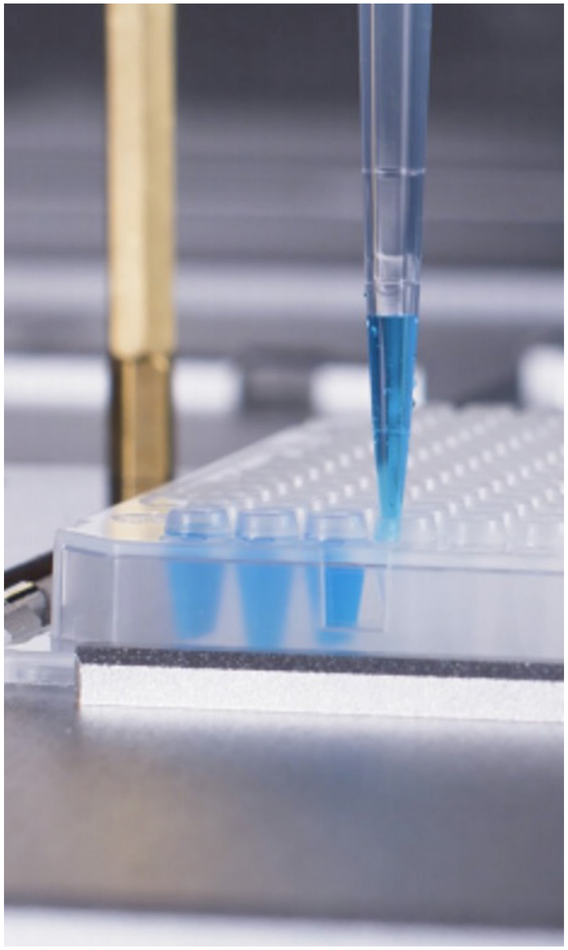


CHAPTER 3

How to Choose the Right Automation Platform for You

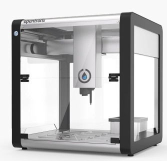
A range of vendors and their various robotic systems are available to automate PCR.

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].



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

Liquid Handler



Thermocycler

Temperature Module

Liquid Handler

The workhorse of automated systems for use in PCR 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

At its core, the thermocycler is a hot plate that can cycle through the three different temperature stages of PCR. Thermocycler modules can be incorporated into some automation units, such that there is a seamless, fully automated transition from plate setup to thermal cycling.

Temperature Module

Used optionally to support varying temperature requirements of reagents and enzymes used in PCR setup.

APPRECIATING THE FULL COST OF AUTOMATED SYSTEMS

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. Often the first aspect researchers consider when selecting automation vendors is the cost of the robotic instrument itself; however, there are several other costs to consider when comparing automation options. 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 PCR.

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. They may not fit well in your existing lab setup and acquiring additional bench space can come with an added cost.

Transition Costs

After delivery and installation, labs need to adapt their manual protocols to their new devices. This transition will involve changes to workflow, standard operating procedures, laboratory information system updates, 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, a PCR 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 consumables, meaning expensive new purchases, and lost time to adjusting workflows and protocols. Some systems require specific labware that may or may not be more costly than what the lab is already using. In contrast, a flexible system could be adapted to utilize the lab's preferred components, avoiding the purchase of new components and lost time to incorporate them into the lab's procedures.

Further, some systems require the use of proprietary reagents or consumables, 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 PCR performance
- Increase throughput
- Reduce costs

Automation can improve PCR performance and increase throughput while reducing costs. Robotic systems can be incorporated upstream and downstream of PCR workflows as is appropriate for a laboratory's needs, or entire end-to-end processes can be automated with a comprehensive workstation. In fact, Opentrons offers several automated solutions, including [the Opentrons PCR setup workstation]().

Automated performance 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 as more automation vendors have surfaced to serve laboratories' needs.

CONCLUSION

Next Steps in Automating Your PCR

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

PRODUCT **The Opentrons PCR Workstation**

BLOG **Assessing Good Quality DNA**

BLOG **PCR Sample Prep Automation On A Budget**

BLOG **Opentrons User Interview: Gina Faraci of Keck Medicine, University of Southern California**

PDF **Viscous Liquid Handling**