Automating clinical NGS: Successes and opportunities for genetic testing

Key learnings

The advantages of automating NGS and the potential of automation to help facilitate routine clinical adoption of NGS-based tests.

The challenges and gaps that remain in current NGS automation technology, and what is needed to overcome them.

Understand the economic value of scalable automation.

Understand the meaning of true end-to-end NGS automation, from nucleic acid extraction to bioinformatics, and learn about current applications where automation is being used successfully, including oncology.

Introduction

Molecular diagnostics continues to evolve at a record pace, with the introduction of new technologies and emerging clinical applications driving consistent growth. Sequencing the genome to detect genetic mutations for clinical diagnostics and precision medicine has traditionally been carried out using older single molecule electrophoretic sequencing, such as Sanger. These low- and medium-throughput sequencing technologies dominated the clinical arena until the development of newer techniques such as massive and parallel sequencing, also known as next-generation sequencing (NGS), in the late 1990s.

The high throughput and accuracy of NGS technologies enable the simultaneous screening of multiple genes from a patient sample, providing information to clinicians on genetic mutations that could serve as prognostic and predictive biomarkers of disease, for example. The cost of sequencing was prohibitive for many laboratories when NGS originally emerged, but the sample sequencing costs of using modern genetic testing in medical applications has decreased significantly as the scale increased in recent years. The National Human Genome Research Institute (NHGRI) determined that in 2006, to sequence a human genome it cost researchers around $10,000, which fell to $1,000 in 2016. Today, it is now about $600.[1] With this decreasing cost, NGS applications have the potential to move outside of core laboratories into the routine clinical setting.

Although it continues to improve, the long duration of NGS sequencing runs can be a barrier against the rapid turnaround times required for clinical sequencing laboratories.[2] For example, a study of the applicability of genomic analysis to routine cancer diagnosis in the United Kingdom (UK) revealed that fully manual laboratory processing for NGS resulted in a turnaround time of as much as six days from request to report.[3]

Additionally, for NGS to be adopted routinely in clinical practice, quality control is needed to ensure results are available, actionable and reproducible, with a reasonable turnaround time. Quality control checkpoints for all stages of the NGS workflow (Figure 1) are crucial in routine laboratory practice in order to achieve high quality sequencing results. Although much progress has been made toward reliable and standardized methodologies implementation, opportunities exist for continued improvement.

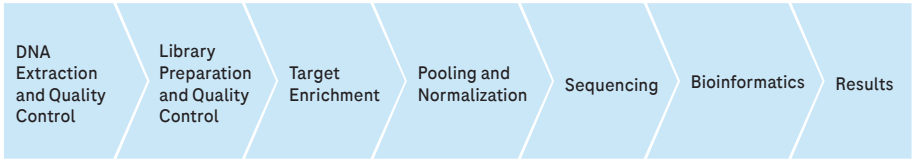

Figure 1: Main steps of the NGS sequencing workflow.1) Sample pre-processing, where nucleic acid must be extracted and purified from the sample. 2) Library preparation, which yields a population of nucleic acid fragments of defined lengths with defined oligomer sequences at the 5' and 3' ends. 3) Target enrichment, where DNA sequences are then either directly amplified or captured. 4) Normalization is completed by using magnetic beads or by dilution with the aid of a fluorometer and then pooled into one tube. 5) Sequencing, usually DNA, RNA or methylation sequencing. 6) Bioinformatics analysis, where sequencing data is processed to generate actionable information. 7) Results, where data is presented to clinicians/patients in a report.

Introduction (continued)

Important developments in molecular technologies have often been catalyzed by the automation of highly complex and time-consuming workflows, such as PCR and, in more recent years, NGS.

Laboratory automation is widespread in the life science and pharmaceutical communities. It speeds up laborious and repetitive tasks, improves lab performance measures by reducing human errors and helps to optimize productivity. Adopting an automated NGS workflow gives clinical laboratories tools to address the growing demand for more molecular testing,[4] especially in fast developing fields such as oncology. Automation is, therefore, increasingly seen as a key solution that is opening doors to the transformative potential of NGS technology, allowing laboratory staff to focus on the science, and move precision medicine in healthcare forward.

This paper reviews the advantages of automating NGS for clinical diagnostics, focusing on oncology as an example of where automation is being successfully implemented to advance genetic testing. It discusses the industry need for a true end-to-end NGS automation approach and considerations for lab directors looking to implement an automation strategy.

The benefits of automation

There are several reasons that a lab might choose to implement automation for NGS workflows. Human resource is a valuable commodity, and the skill and time required to complete steps manually places a considerable burden on the lab. Regardless of operator skill there is always a risk of manual processing errors and inter-operator variability, and the need for highly reproducible results is especially crucial in the clinical setting where successful patient treatment depends on accurate results from genetic tests. There is, therefore, a clear patient benefit for reducing handling error rates in NGS by using automation strategies.

Other key benefits of NGS automation for the clinical lab include:

- Quicker turnaround time for sample to result – patients can receive diagnosis and therapy faster
- Sample throughput and scalability
- Improved sample tracking and traceability
- Productivity outside human working hours (nights and weekends)

Automating library preparation

The main challenges facing the accessibility of NGS for clinical diagnostics include protocol complexity and cost, both of which are mainly entrenched in time-consuming library preparation steps.

The quality of sequencing data depends on the performance of the library preparation chemistry and due to the complicated library preparation workflows, the process is susceptible to human errors. Sample cross-contamination within runs is a potential problem in library preparation as libraries are usually prepared in parallel,[2] and carryover contamination can occur between runs.

Library preparation can also be incredibly time-consuming when carried out manually, often taking several days and creating a bottleneck in the NGS workflow. Streamlining the costs of library preparation, primarily derived from a combination of laboratory equipment, trained personnel's hands-on time and reagent costs, will contribute to NGS becoming more accessible and cost-effective in the clinical setting.

By automating library preparation, clinical labs can perform complex protocols with high reproducibility, continuous operation, reduced error rates, and decreased cost per sample by reducing hands-on time.

However, although incorporating automation into NGS library preparation workflows presents clear advantages, not every laboratory is poised to implement this strategy. A laboratory's capacity to implement NGS automation will depend on varying requirements across research, core sequencing, and diagnostic laboratories. Other factors that may influence efficient automation strategies could be cost per sample, investment costs, space, resource availability, as well as staff training.

The upfront cost of automated systems, and a lack of experience with automation, can deter smaller or clinical laboratories from deploying automation. Many labs also consider themselves outside the remit of automation due to lower NGS throughput, but even laboratories processing as few as eight samples per run can benefit from automating NGS library preparation. By anticipating the benefits versus the challenges of adopting automation, clinical lab directors can make informed decisions.

The automation status quo

The demand for more accurate, error-free data, as well the development of more accessible low- to mid- throughput liquid handlers at lower price points, are driving the NGS automation market. The global sample preparation market size is expected to reach around US$ 9.4 Billion by 2026 and is growing at a compound annual growth rate (CAGR) of around 5.2% over the forecast period 2019 to 2026, attributed to the shift from manual to automatic sample preparation methods.[5]

Compared with automated DNA extraction,[6] which has seen a spike in popularity due to the COVID-19 testing market, the library preparation automation segment is relatively fragmented, with many liquid handling platform providers and microfluidics vendors competing for market share. This fragmented market offers laboratories flexibility to find a solution that fits their needs.

The benefits of automation (continued)

The variety of systems range from simple, low-throughput liquid handling robots, to large and expensive high-throughput systems that require considerable technical expertise to operate (Figure 2).

These categories of automation have rapidly evolved to meet the needs of a range of laboratories, from large core sequencing centers to smaller hospital laboratories.

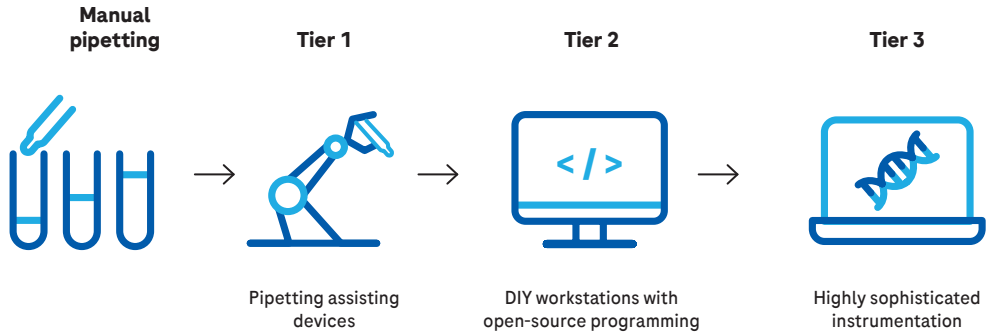

Figure 2: Different categories of liquid-handling robots. Automated liquid-handling systems range from highly sophisticated workstations (Tier 3) to DIY workstations with open-source programming (Tier 2), or simple pipetting assisting devices (Tier 1). Adapted from[7] in accordance with Creative Commons Attribution 4.0 International License.

However, despite the growing interest in streamlined clinical NGS workflows, there remains a widespread lack of end-to-end solutions that are designed with the clinical lab in mind. To facilitate uptake of NGS in clinical labs there is a need for this level of automation, from nucleic acid extraction, library preparation, to bioinformatics.

Data processing and reporting using integrated workflow management lags behind in NGS, particularly in the clinical setting. The turnaround time of tests is crucial for effective patient care and test viability, and hinges on the availability of comprehensive and meaningful clinical reporting of NGS data. Classifying, interpreting, and reporting thousands of variants to achieve clinically insightful outcomes requires time, and the incompatibility of available bioinformatics tools with commonly used computer operating systems further compounds the problem.[8] There is, therefore, a need for automated solutions that facilitate reporting of multi-parametric NGS data and workflow management, that allow clinicians to draft clinical reports automatically from their existing laboratory information systems.

In addition, users are often required to develop automated solutions themselves which requires special expertise in automation programming and can be a significant barrier for labs. The availability of pre-developed methods combined with a system that enables labs to readily optimize the workflow for their needs, would represent a significant step forward for adoptability of NGS automation in the lab.

One aspect of automated liquid-handling that still has room for improvement is evaporation control, which is particularly relevant to the volumes handled in genomic workflows.[9] The reality of fully automated protocols requiring no user intervention is still yet to be achieved, limited by the uncertainty of low liquid volumes resisting evaporation inside the workstation.[7] Some modern systems have been designed to mitigate the effects of evaporation, for example by using sensors to monitor external conditions,[10] or by incorporating a reservoir lid that the robot can manipulate to reduce the evaporation of liquids such as ethanol. However, there is more engineering work required to overcome this obstacle in automated liquid-handling.

Deck size can also be a limitation in current liquid handling systems, particularly for long or complex workflows that require many consumables. Instruments with cabinets underneath the deck that the robot can access or integrated robotic storage systems are beginning to address this challenge, and help to minimize the instrument footprint, which is important for clinical labs with limited space.

Success story of NGS automation: Oncology & Genetics

It has been known for years that cancers are linked to genes and genetic mutations.[11] Genomic sequencing technology has allowed clinicians to unveil and better understand the mutations or genes that are involved in certain cancers. Armed with this knowledge, physicians may be able to tailor treatment options to the individual patient. NGS technology has provided the ability to generate a wealth of data, advancing this clinical understanding further.[12]

As the availability of genome sequencing has increased, it has begun to influence treatment decisions on a real-time basis, rather than providing post-hoc explanations for serious medical problems.[11] Genomic approaches are finding links to diseases that do not match conventional clinical outlooks or presentations, as well as providing insights into the prognosis and treatment options for these diseases. With this better understanding comes better results for patients and those affected by cancer or genetic disorder.

Although using NGS as a diagnostic tool can be costly, a holistic view of the total cost of care is important to keep in mind. A study looking at infants with suspected monogenic disorders found that the cost of diagnosis was lower when using exome sequencing (AU$ 5,047) than that for standard care diagnosis (AU$ 27,050).[13] Since a large portion of the costs of genome sequencing can be attributed to the laboratory personnel,[14] further decreases in the cost per sample can arise from increasing sample processing capacity, or by reducing hands-on time, and therefore costs, to process these samples.

Some oncology applications have seen particular success in automating genetic tests for prognosis, diagnosis, and treatment decisions. One study compared manual and automated somatic (lung/colon panel) and germline (*BRCA*1/2) library preparation protocols for NGS analysis from FFPE and peripheral blood samples. The authors reported an improvement of NGS standardization and increased sample throughput, as well as optimization of reagents and management, which resulted in a doubling from one to two sequencing runs per week.[15]

Another study subjected RNA samples to comparative manual and automated library preparation methods. It indicated a high degree of similarity between libraries generated manually or through automation, with a significant reduction in both hands-on and assay time from a 2-day manual to a 9-hour automated workflow. This demonstrated that automated workflows can be of great benefit to genomic laboratories by enhancing efficiency of library preparation, reducing hands-on time and increasing throughput potential.[16]

Automation of NGS analysis for clinical workflows is vital for the timely delivery and actionability of results and cost-effectiveness of NGS testing in oncology. A group in Chile recently optimized and validated a hybridization-based target enrichment workflow with multiple automated analyses capable of detecting variants in 25 genes (single nucleotide variants (SNVs) and small insertions and deletions) with 100% sensitivity and specificity.[17] To address the shortage of health professionals trained in bioinformatics, the entire workflow, including quality control of sequencing data and calling for somatic variants, was automated and made available. It is expected that such automation of NGS analysis could facilitate the adoption of precision oncology, particularly in low- to medium-income regions such as Latin America.

A view from the lab

Roche hosted a discussion forum in May 2020 to assess the needs of different lab professionals (n=21)[a] in both research and clinical settings, to gain insight into priorities for NGS library preparation automation solutions. There were a number of features and benefits of a putative automated liquid handler that appealed to both groups, including reduced hands-on time, shortened turnaround time, and reduced errors. Table 1 highlights some priorities specifically outlined by the professionals in clinical laboratories (n=10).

Feature	Benefit	Comment
Flexibility	The ability to run various sample quantities, volumes, and test types allows labs to respond more quickly and easily to changing needs/workload	*"The usability for oncology, human genetics/ molecular genetics and infectious diseases reflects perfectly our needs in the routine diagnostic lab."*
Customizable workflows	The option to create custom programs is appealing to a range of clinical labs	*"The option of creating custom programs by the user is very good and much needed in a diverse lab setting."*
Stability of reagents over time	Reduces waste and therefore costs	
Simple software with step-by-step guidance	Reduces training time required and potential for errors. Speeds up process by guiding lab staff through each step	*"I think the step-by-step directions are very beneficial especially when training new hires."*
Sample tracking and workflow management	Users can easily trace samples and patient results	
Full end-to-end automation: One system automation for library preparation, target enrichment, quantification, thermocycling and pooling	Less hands-on time required, simplifies workflow, faster turnaround time, automates tedious manual processes, reduces potential for errors, frees lab staff for other tasks, increases lab's testing capacity	*"The fully automated features including the library prep, thermocycling and pooling are good."*

Table 1: Features and benefits of automated liquid handling systems for next-generation sequencing (NGS) identified by clinical laboratory professionals.

a. Given the small sample size, results are interpreted based on qualitative research and should be regarded as directional rather than predictive or definitive.

A view from the lab (continued)

Clinical labs also indicated the importance of full laboratory information management system (LIMS) connectivity and ease of integration with existing infrastructure, with support and training from the manufacturer. In addition, several respondents highlighted that an automation system would need to fit into their lab footprint. These results indicate that clinical labs are not only concerned with liquid handler performance and its ability to advance their NGS testing, but automation systems must meet the practical needs of the lab.

Concluding remarks and future potential

The growing trend for precision medicine, as well as the decreasing cost of sequencing and greater collaboration between industry, clinical researchers, and regulatory bodies, have paved the way for NGS uptake into the clinical space. Automation methods have facilitated the scale-up of NGS and enabled greater standardization, reduced labor costs, reduced hands-on time, and optimization of reagent use in routine workflows.

Library preparation is often the main bottleneck in the NGS workflow and is one of the most hands-on, error-prone, and time-consuming steps. To optimize the process, automated library preparation is becoming an increasingly important tool in NGS. Automation can reduce human error and overall sequencing costs, while minimizing the variability found in manual processing and providing valuable walkaway time for lab staff. Space remains in the market however for a system that can automate the entire workflow, from reagent barcoding and preparation, library preparation, quality control (QC), and target enrichment on deck, through to bioinformatics with integrated workflow management software that can track samples and generate user-friendly reports for clinicians.

The NGS space is dynamic and is set to develop considerably over the next decade. For instance, upcoming developments in the regulatory landscape will influence the uptake of NGS in a routine clinical setting. The additional quality requirements for genetic tests that are mandated by the Verifying Accurate and Leading-edge IVCT Development (VALID) Act in the U.S., and the new European Union (EU) Regulation (EU 2017/746), highlight the important role of NGS automation for generating highly reproducible, standardized and compliant results.
NGS reimbursement policies and coverage decisions will also be impacted by the ability of clinical labs to comply with new regulations.

For more information on automating your NGS workflow, please visit, https://sequencing.roche.com/NGSAutomation.html

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