STARLIMS™

Integrating Next Generation Sequencing into Mainstream Laboratory Testing

In this day and age, it is important to stay ahead of the curve and Next Generation Sequencing (NGS) are innovative sequencing platforms that can perform hundreds to millions of sequencing reactions in parallel, while simultaneously analyzing the resulting sequencing data. Recently, the Centers for Medicare & Medicaid Services (CMS) recently finalized the decision to cover diagnostic laboratory tests using NGS for patients with advanced cancer; a decision that will undoubtedly accelerate the adoption of NGS in clinical diagnostics.

Scientists started sequencing DNA several years before laboratory information management systems (LIMS) were commercially available in 1982 to help control laboratory processes, track samples, and laboratory workflows and data. In this article we will discuss how work with DNA has advanced to Next Generation Sequencing and how modern laboratory testing can help streamline the process of sequencing DNA, by offering higher quality diagnostics testing and a more effective process.

Forty years ago DNA sequencing was a labor-intensive, manual process that used radioactive materials and only generated sequences of isolated DNA molecules. The sequence of base pairs for at least a small stretch of the DNA had to be known in advance, and was used as the primer that would act as the initial binding site for the sequencing enzymes, which would generate the previously unknown sequence. The end of the new sequence would be used as the primer for the next round of sequencing. Using this approach, known as Sanger sequencing, an entire gene could be sequenced a few hundred base pairs at a time. The average gene is 10 to 15 thousand base pairs long, so it's easy to appreciate how time consuming this was, even after automation of the technology in 1987.

The first platforms for Next Generation Sequencing (NGS), which emerged at around the start of this century, built upon the Sanger method (amplicon sequencing), but are able to carry out hundreds to millions of sequencing reactions in parallel, together with the analysis of the sequencing data that is being produced. As well as generating massive amounts of data, today's NGS platforms are incredibly fast and relatively cheap in comparison to earlier applications of DNA sequencing. The costs are continually falling thereby facilitating the use of NGS, which is a form of automated DNA sequencing, for many new applications that just 10 years ago would have been cost-prohibitive. Today, NGS has moved into the healthcare arena, and is used increasingly for clinical diagnostic applications (e.g., diagnostics testing). Commonly used NGS processes, including whole exome sequencing, DNA target-based sequencing, RNA sequencing, and chromatin immunoprecipitation sequencing, are behind the development of innovative processes and discoveries in both research and patient-centered settings.

NGS advances the realm of Clinical Diagnostics Testing

In the clinical diagnostics arena, NGS is being exploited in areas including inherited disease diagnosis, oncology, genetic counseling, and infectious disease management. And it was for an inherited disease that the first NGS tests were FDA approved, specifically, Illumina's MiSeqDx Cystic Fibrosis 139-Variant Assay and MiSeqDx Cystic Fibrosis Clinical Sequencing Assay. In clinical oncology settings NGS is used for tumor profiling to provide insight into disease prognosis and to aid in therapeutic decision making. NGS is also underpinning the development of personalized medicine approaches that use NGS-based companion diagnostic assays that are paired with drugs that have been developed and approved for use only in subsets of patients with specific genetically defined tumors. Accordingly, this type of technology may also help better define gene regulation.

The first NGS companion diagnostic to win FDA approval was Foundation Focus' CDxBRCA, which aids in identifying patients with BRCA-mutated ovarian tumors for whom treatment with Clovis Oncology's Rubraca (rucaparib) is being considered. The next two NGS companion diagnostics approved by FDA were Illumina's Praxis Extended RAS Panel for identifying colorectal cancer patients who are eligible for treatment with Vectibix, and Thermo Fisher Scientific's Oncomine Dx Target Test, for selecting non-small cell lung cancer (NSCLC) patients with specific gene mutations.

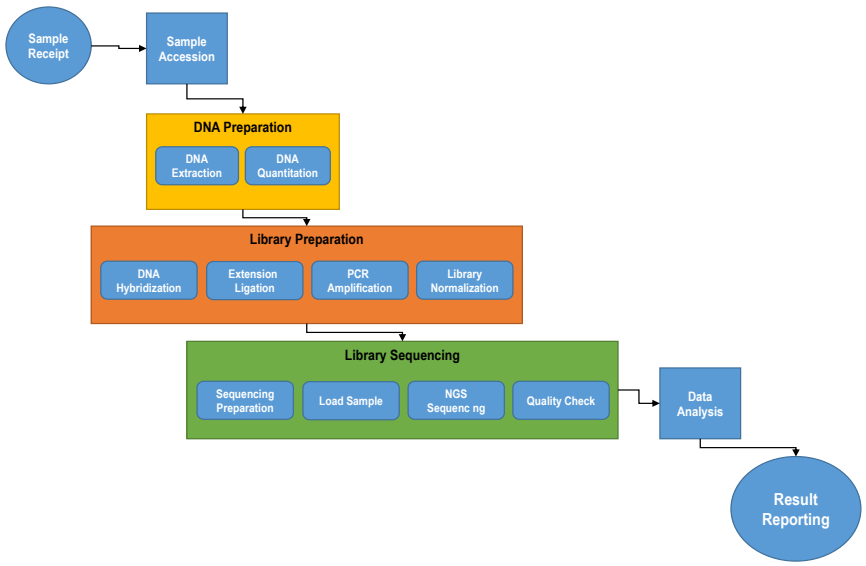
FDA-approved tests have a specific, developed and validated protocol to follow. But even the most straightforward FDA-approved NGS assay protocol involves a complex workflow with multiple steps and procedures. A basic NGS workflow may follow a path through multiple laboratories that carry out different functions including sample preparation, DNA library preparation and amplicon sequencing. Upstream of this multi-facility workflow samples will be received and logged, and downstream the resulting data must be analyzed prior to release of the results.

Employing a LIMS system to manage these workflows can greatly benefit laboratory efficiency and proficiency.

For laboratories that are carrying out NGS testing and assay development, setting up and adjusting complex workflows can be time-consuming and complicated, so having pre-existing workflows that follow some of the more standard procedures is advantageous. A greater complexity is introduced when the laboratory is performing NGS testing for clinical applications of DNA sequencing. In many cases FDA approved tests come with established performance characteristics, standards and controls to demonstrate the validity of the results. With some FDA approved tests, such as Illumina's MiSeqDx Cystic Fibrosis 139-Variant Assay, there is still a need to view and interpret the results in conjunction with additional laboratory and clinical information. With the exponential development and release of NGS assays for clinical purposes, specific guidelines have been developed surrounding the procedures associated with NGS testing and reporting of results.

Regulatory Bodies Work to Catch Up to NGS

In the United States clinical laboratories are subject to oversight from several governing bodies. The Centers for Medicare and Medicaid Services, regulate laboratory testing via the Clinical Laboratory Improvement Amendments (CLIA). Then there are state level requirements that may be more stringent than those set by CLIA. There are also laboratory professional organizations such as the College of American Pathologists (CAP), which sets best practices guidelines for clinical laboratories. And as molecular technology has advanced, organizations like the American College of Medical Genetics and Genomics (ACMG) and the Association of Molecular Pathology (AMP) joined the field.



Abbott

In 2015 ACMG and AMP released a joint guidance on the interpretation of genetic testing for clinical diagnosis[1]. These guidelines strongly recommended that clinical molecular genetic testing be performed in CLIA-certified laboratories and included specifics on who can interpret the results1. These guidelines also stated that there is a need to carry out results confirmation, where sequence variants are considered to be pathogenic or likely pathogenic as described by the guidelines[1]. This publication referenced a previous set of guidelines released by ACMG (2013) specifically for NGS (automated DNA sequencing) which recommended methods for confirmatory testing and provided guidance for development, testing and validation methodologies, and reporting standards.[2]

Separately from the ACMG, in 2014 CAP added 18 new laboratory accreditation checklist requirements to the CAP molecular pathology checklist[3]. The requirements apply to NGS-based assays across multiple disease areas, including inherited disorders, molecular oncology, and infectious diseases[3]. These requirements are split between wet lab and dry lab and address many topics surrounding NGS, including confirmatory diagnostics testing[3].

There are LIMS systems that are starting to accommodate the complexity of NGS workflows involving multi-directional sample processing and multiple disciplines within the laboratory. But they are not necessarily configured to manage workflows from start to finish. In order to achieve the final result NGS is heavily reliant on data analysis which is dependent on the initial assessment of amplicon sequencing software. Initial analysis requires determining the exact sequences of the individual copies of nucleotide strands, aligning the short reads into overlapping longer reads, and then comparing the individual reads to identify variants, while taking into account signal strength, errors and other quality metrics. Clinical NGS laboratories are expected to set thresholds for the variants identified and the interpretation of whether these variants are clinically significant or benign.[2] There is also the expectation that high quality standards are maintained for laboratory-developed diagnostics testing, and more specifically, the assurance of low false positive rates.

According to the ACMG clinical laboratory standards for next-generation sequencing, "it is recommended that all disease-focused and/or diagnostic testing include confirmation of the final result using a companion technology."[2] The expectation is that, moving forward, only the most experienced NGS laboratories using well established and proven algorithms could consider eliminating confirmation testing with orthogonal technology. Therefore, in lieu of performing the extensive validation required for every type of variant that may be called using NGS, laboratories turn to Sanger sequencing as one of the most commonly employed technologies for confirmation of germ line DNA testing. Sanger sequencing is particularly useful given that "it is currently recommended that all disease-focused testing of high-yield genes include complete coverage in each patient tested,"[2] as would be found in gene panels and other target enrichment tests. Meanwhile, other assays may be validated using replicate testing, often seen in tumor tissue analysis or mosaic variants, or using companion technologies such as fluorescent in situ hybridization (FISH).

LIMS Meet NGS Workflows

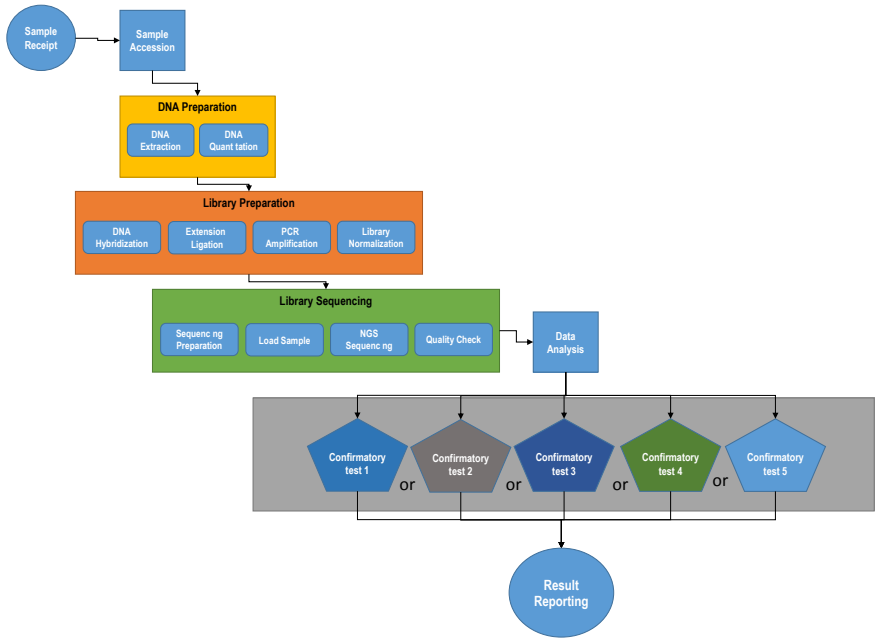
Many laboratories that have implemented a LIMS to manage their NGS workflows have done so up to and including the recording of variant call results. The ultimate aim, whether they are using one LIMS or combining multiple systems, is to use informatics technologies to manage the complete NGS process. From sample extraction, library creation, and amplification, through to automated DNA sequencing, laboratories are also now starting to link data analysis into their workflows. However, confirmatory testing is often not included in those workflows. Rather, confirmatory tests are commonly treated as separate assays and may not even be included within the processes of the LIMS that is managing the complete workflow upstream of confirmatory testing. Yet ACMG recommends that confirmation testing should be planned in advance of the need[2], while the CAP checklist states that a laboratory has to have policies in place for carrying out required confirmatory testing.[3]

Also, when providing projected assay turnaround times, laboratories should take into account the time needed to carry out confirmation testing. And in instances where preliminary results are released prior to the completion of confirmatory testing, it is recommended that the report state that confirmatory testing has yet to be carried out. For example, "the following results have not been confirmed by an alternative method or replicate test."[1]

Utilizing LIMS to meet Regulatory Requirements in NGS

For Example

Following the CAP/ACMG guidelines, a 20 gene panel would require the detected mutations and novel variants that pass the quality requirements to be confirmed by Sanger sequencing. In this hypothetical situation the assumed rate of patients with detected mutations and novel variant calls is 10%, and of the 20 genes in the panel, the confirmatory tests fall into 5 possible protocols. Therefore, a high throughput laboratory that is running hundreds of patient samples a day could require 10% of those samples to undergo some sort of confirmatory testing. Taken further; out of 500 patient samples 50 would require one of 20 possible confirmatory tests to be performed, following one of 5 possible methods.



To accommodate confirmatory testing within a LIMS rules can be set in place for the next test or the reflex test based on the results that are returned. Ideally, this will be part of a seamless workflow that doesn't require the resubmission of new samples into the laboratory and manual oversight of methodology distribution. Instead, it should follow established rules, automatically redirect the testing to the confirmatory methodology, and await the confirmatory result prior to final report release.

High throughput laboratories that use multi-gene cancer panels to assess patient samples face particularly challenging workflows. Sanger sequencing (amplicon sequencing), which is the most common method used for confirmatory testing, allows for different primer pairs to be used on the same platform, but it is unlikely that all the possible primer pairs associated with an NGS gene panel could be assessed together. Using a LIMS to manage the number of samples that may require confirmatory testing would be much easier when there are already rules in place for directing samples to the different tests, and recognizing which tests can be performed simultaneously, rather than having to manually add tests and try to link them to the original samples after the fact.

NGS methodology has allowed the development of advanced clinical tests that can inform more targeted, effective patient care. However, the complexity of NGS-based assays and results analysis in a clinical setting means that confirmatory testing is likely to be required. It should be a natural progression for both clinical and non-clinical testing laboratories to include confirmatory testing into their LIMS processes. Workflows can be configured as new tests are developed, and additional validation methods are added.

Exploiting a LIMS to combine laboratory methodologies of sample testing based on pre-defined logic can only help to improve to laboratory efficiency. And with NGS methods evolving in process and data complexity, the ability of a LIMS to automate and manage workflows, processes, and results analysis and reporting, will become even more invaluable, both in clinical and research environments as well as additional applications of DNA sequencing.

The benefits are manifold. Automating rules-based confirmatory test method selection, sample scheduling and results reporting can realize significant cost and time savings, while reducing manual data entry and the potential for errors. These processes are facilitated through the automated DNA sequencing ability of NGS. LIMS automation also reduces the likelihood that confirmatory testing for individual samples isn't accidentally overlooked. Using a single platform to manage all samples and coordinate first-round and confirmatory testing and results reporting, offers a more complete picture and data repository, accessed through a single point of access and potentially delivering confidential reports to physicians and clinicians faster.

1. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015; 17: 405-424.

2. Rehm HL, Bale SJ, Bayrak-Toydemir P, et al. ACMG clinical laboratory standards for next-generation sequencing. Genet Med. 2013; 15: 733-747.

3. Aziz N, Zhao Q, Bry L, et al. College of American Pathologists' Laboratory Standards for Next-Generation Sequencing Clinical Tests. Arch Path Lab Med. 2015; 139: 481-493.

informatics.abbott

ABBOTT INFORMATICS
Tel: +1 954 964 8663
4000 Hollywood Blvd, Suite 333 South, Hollywood, FL 33021-6755 USA

UNITED KINGDOM
Tel: +44 161 711 0340

GERMANY
Tel: +49 2302 915 245

FRANCE
Tel: +33 1 61 37 02 00

SPAIN
Tel: +34 91 663 67 64

NETHERLANDS
Tel: +31 72 511 8100

ASIA PACIFIC
Tel: +852 2793 0699

LATIN AMERICA
Tel: +1 954 964 8663

CANADA
Tel: +1 888 455 5467

AUSTRALIA
Tel: +61 3 9670 0678

INFORMATICS

Abbott