Guide to implementing NGS

Dvysr®

Contents

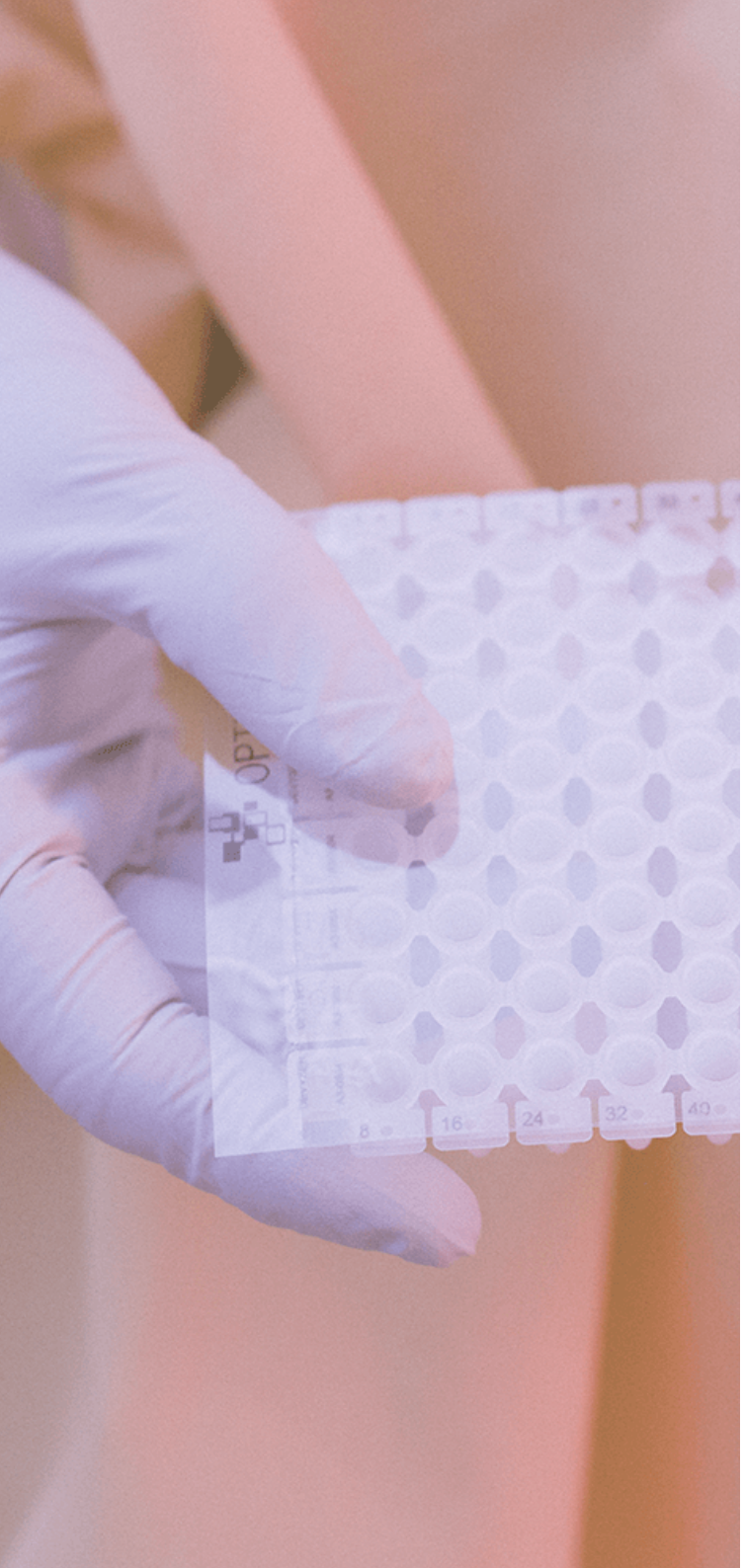
Simpler, faster, better

■■■ When planning to adopt a new NGS test in the clinical laboratory, many questions and practical considerations arise. Will introducing this new test increase or decrease complexity in the lab? Which activities will be impacted in the wet-lab and how will this affect cost-efficiency? What equipment, materials and reagents do I need to get started? How much training will be needed? Whether you are new to NGS or already run some NGS diagnostics routinely, this guide addresses frequently asked questions and highlights key factors to consider when adding a new NGS test to your offering. Learn how recent advances in NGS solutions can streamline testing workflows in your lab—saving time, improving the quality of results, and increasing efficiency. You will also find links to useful resources, such as our sequence coverage calculators and NGS checklist tool.

"Using the Devyser Thalassemia kit, we can reduce both response time and costs. One single assay provides all the information we need."

Dr. Veysel Sabri Hancer

→ contents



NGS in routine diagnostics

■■■ Next generation sequencing (NGS) is transforming clinical genetic testing with increased diagnostic accuracy, sensitivity and efficiency. With the power to sequence millions of DNA strands in parallel, NGS enables more genes and loci to be analyzed in a single test, compared to more traditional methods. Recent innovations have also made it possible to combine detection of single nucleotide variants (SNV), indels and larger deletions (CNV) in a single sequencing run. These developments have made it feasible to use NGS as a primary test method for diagnostic cascades, shortening the time it takes to get a definitive result and reducing the need for multiple primary methods like Sanger sequencing, GAP-PCR, MLPA, ARMS-PCR and other allele-specific detection methods.

At the same time, significant improvements have been made in NGS sample and library preparation. Where once multiple tubes, sample aliquots and clean-up steps were needed to prepare high quality NGS libraries, diagnostic kit providers now offer solutions that significantly reduce hands-on time, sample handling, and the risk of sample contamination and mix-ups.

Together, these advances lower the barriers for NGS diagnostics, and make complex genetic analyses simpler, faster and more reliable.

With today's advanced NGS solutions, routine diagnostics laboratories benefit from:

- Improved reliability and accuracy of genetic tests
- Faster turnaround times
- Easy-to-use kits that simplify laboratory routines
- Reduced risk of user introduced errors
- User friendly data analysis solutions
- Cost-effective results
- Reduction in the number of tests needed to reach a definitive molecular diagnosis
- Capacity for staff to take on other work as procedures require less hands-on time and total turnaround times are shortened
- Decreased risk of errors and the consequent need for repeat testing
- Less specialist training and time required for analysis and interpretation of results

→ contents

What to expect

Staffing requirements

■■■ With the ability of NGS to analyze thousands of genetic markers in a single test, implementing a new NGS test enables a more streamlined diagnostic workflow. Depending on the application, many labs find that there is no net impact on the number of staff required, although some level of retraining will be needed initially and competency maintained for the new method (see Training and assay maintenance).

Breadth of assay technologies

When migrating to an NGS-based diagnostic workflow, in most cases the breadth of assay technologies being used does not change, but the frequency and volume of their use will shift. For example, if NGS replaces an allele specific detection method, Sanger sequencing or GAP-PCR as a primary diagnostic test for a particular disease state, the legacy tests will typically still be needed, but as secondary tests they would be run less often.

Assessing the impact on workflows

When assessing the potential impact of a new NGS test and/or diagnostic routine, the first step is to map out your current testing workflow and compare it side by side with the proposed NGS solution. This will help you spot opportunities to replace or consolidate work streams, compare turnaround times, and understand how the balance of activities in the lab is likely to change.

→ contents

Genetic testing for thalassemia

■■■ Thalassemia is an example application area where NGS can bring significant improvements to the genetic testing workflow by detecting both structural and all sequence variants in the HBA1, HBA2 and HBB genes in a single run.

Current testing strategies

Most genetic testing strategies for thalassemia utilize a range of different assays to detect and characterize mutations in the globin genes at the molecular level. Common assay methods include GAP-PCR and MLPA for deletion analysis, and Sanger sequencing, or reverse dot blot and ARMS PCR to identify single nucleotide polymorphisms.

Following initial hematology tests indicating a possible thalassemia, patient samples are run through an evaluation cascade (Figure 1A). The nature and sequence of tests in the cascade differ depending upon whether alpha or beta thalassemia is suspected.

If alpha thalassemia is suspected, the primary test in the cascade is typically GAP-PCR to detect common deletions. If this returns a positive result, the cascade is terminated. If the result is negative, then successive tiers of testing are carried out until a pathogenic sequence variant is identified. If all testing options are exhausted without a positive result that explains the clinical manifestation, the sample is taken through the beta cascade.

Not only is this strategy complicated, labor intensive and time-consuming; there is an inherent risk that some pathogenic variants will be missed if the evaluation cascade is terminated after a first variant is discovered (Table 1).

Streamlined NGS approach

Utilizing the Devyser Thalassemia NGS kit for the primary test simplifies the evaluation cascade considerably (Figure 1B). The majority of both alpha and beta thalassemia variants can be detected on the first try in a single test. This reduces the frequency and number of samples requiring follow-up testing with secondary methods. The total turnaround time is reduced from several weeks to just a few days (Table 1).

→ contents

Figure 1A

A typical alpha and beta thalassemia evaluation cascade

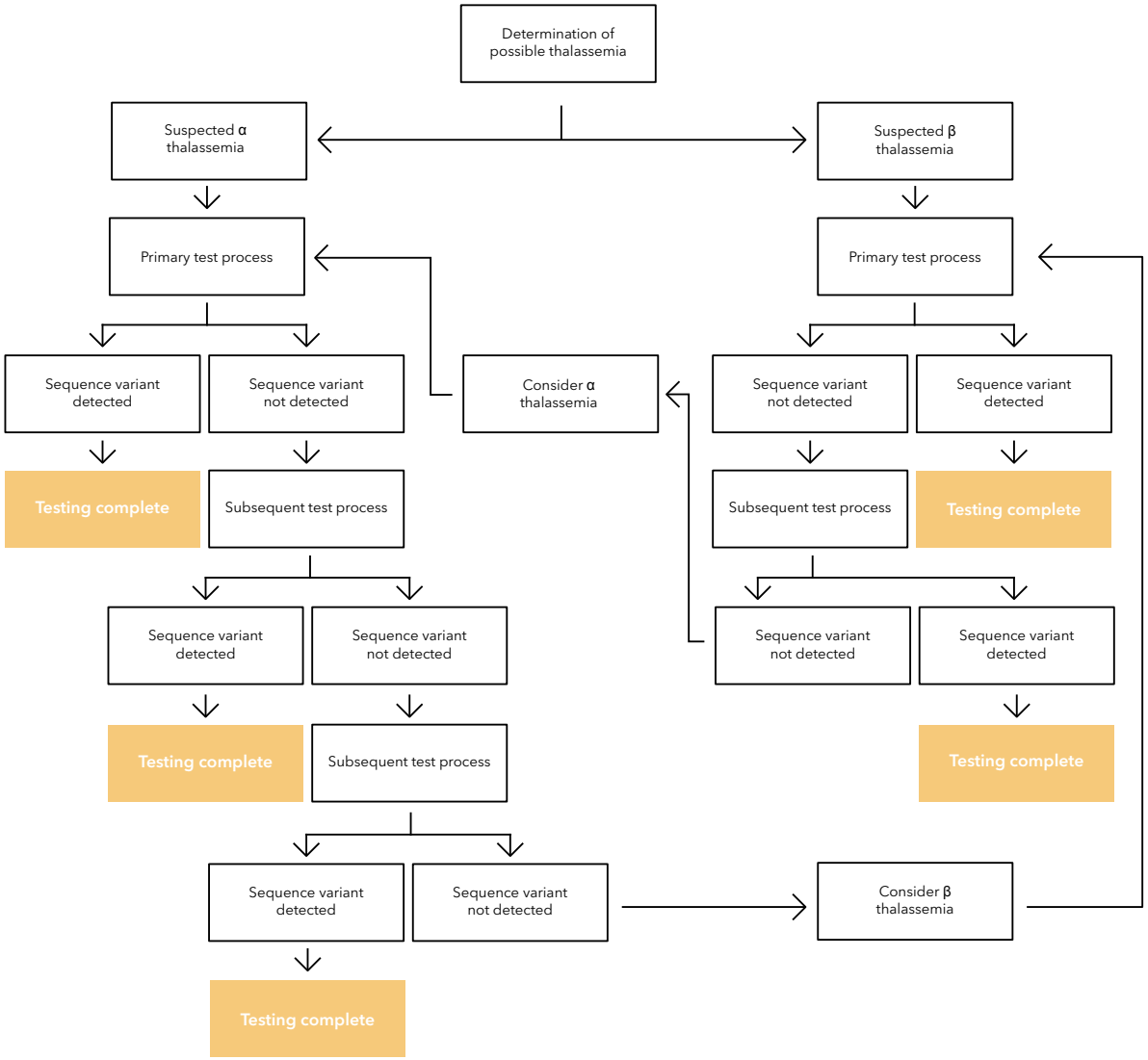
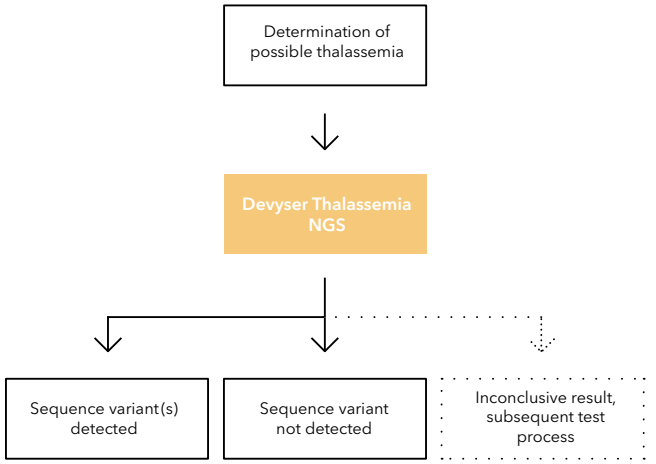


Figure 1B

A streamlined workflow with Devyser Thalassemia NGS



→ contents

Table 1

Thalassemia workflow comparison

Method	No. of tests to completion	Typical turnaround time	Workflow	Analysis and interpretation
Typical evaluation cascade	up to 4	2-4 weeks 	• Complex cascade with multiple work streams and decision points • Many different techniques with more opportunities for error • Resource-intensive and costly to validate, maintain and train operators on multiple assays • Risk of sample contamination and mix-ups when handling multiple tubes and protocols • Multiple quality control measures required to cover all assay methods	• A variety of analysis protocols depending on which assays are performed • Specialist knowledge (e.g. hematopathologist) to interpret and direct each stage of testing to completion • Risk that some variants will not be detected if workflow is terminated after a first variant is found, or if using a targeted detection approach that analyzes for specific genes, loci or variant only
Devyser Thalassemia NGS	1	1-2 days 	• A single primary workflow (NGS) with fewer secondary tests needed • Single-tube assay for simultaneous analysis of HBA and HBB gene clusters • Built-in rapid sample mix-up control • Kit design reduces the need for additional QC assays	• A tailored bioinformatic software pipeline facilitates data analysis • SNVs, Indels and CNVs are rapidly analyzed and intuitively displayed • Robust and reliable detection of deletions and duplications by two methods • High coverage density for precise deletion mapping with HBA1, HBA2 and HBB genes fully sequenced for comprehensive detection and reduced risk of missed variants and false negatives

→ contents

The NGS workflow

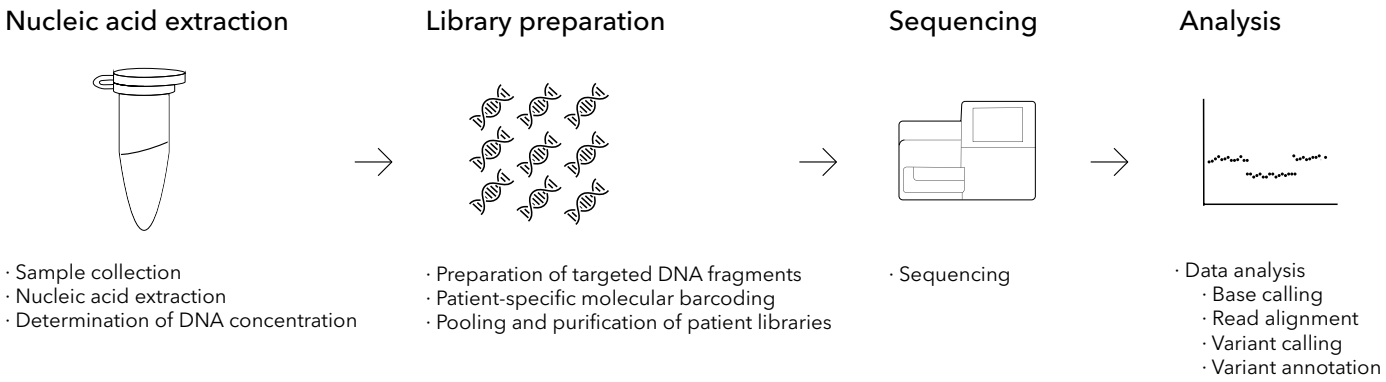


Figure 2:
The typical NGS workflow has four main stages

Nucleic acid extraction

■■■ Consistent preparation of high quality nucleic acid is the starting point for accurate and reliable NGS results. For clinical applications, nucleic acid extraction kits should offer high performance to ensure availability of pure DNA. A high-sensitivity DNA quantification kit and suitable detection system are recommended to facilitate accurate quantification. Accurate and reproducible results are typically obtained when using fluorometric DNA quantitation methods, such as Qubit.

Library preparation

Library preparation entails preparing targeted DNA fragments labeled with patient-specific molecular barcodes to allow identification during data analysis. Since library quality is fundamental to NGS success, it is advisable to review and compare NGS library preparation protocols before making your selection. A protocol with few steps and a minimum of hands-on time can reduce the need for time-consuming quality assurance and error proofing without compromising library quality.

Sequencing

The sequencing step is straightforward, Once the sequencing chip or flow-cell has been prepared and the run initiated, the process is fully automated. Run times vary depending on the sequencing system, the number of samples being run and the protocol set-up, but can range from hours to several days (for example, see Table 1).

Data analysis

NGS generates large quantities of raw data, which must then be processed and analyzed to identify the presence or absence of genetic variants and their functional annotations. For clinical applications, regulatory compliance of the analytical software is also essential.

To help routine diagnostics labs transition to NGS, Devyser has partnered with leading analytical software providers to enable Devyser NGS applications on popular analytical platforms such as Amplicon Suite.

→ contents

3-step library preparation method

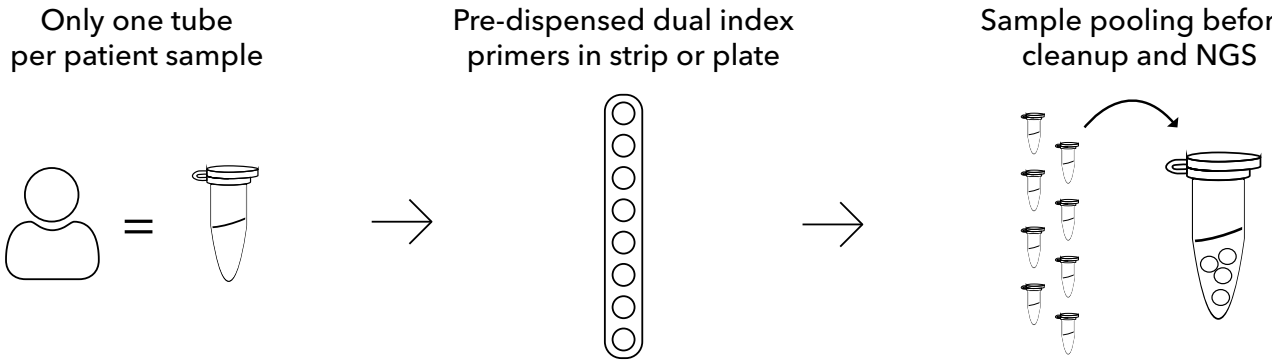


Figure 3:
Simple 3-step library preparation

■■■ Library preparation is typically the most labor intensive, time consuming and tedious step in the NGS workflow. Given how essential high quality libraries are to NGS success, it is important to choose a rapid, robust and reliable library preparation method for clinical applications. Devyser NGS kits have been designed with routine diagnostics labs in mind. With only one tube per patient sample and one tube per library pool during cleanup, Devyser's simple 3-step protocol (Figure 3) speeds up NGS workflows while reducing the risk of sample contamination and mix-up. The technology is fast and easy to use, reducing hands-on time to less than 45 minutes.

Step 1. Library preparation. Add PCR mix and purified DNA to a single tube for each patient sample, and amplify by PCR. Unlike other library prep solutions, there is no need to split the primary patient sample into multiple sub-aliquots. This reduces the amount of labor, facilitates labs to run more samples, and decreases the risk of contamination and sample mix-ups.

Step 2. Library barcoding/indexing. All index primers are pre-mixed and pre-dispensed, minimizing set-up time and the risk for sample mix-up. The highly flexible format allows you to run anything from one to 96 samples simultaneously.

Step 3. Pooling and purification. To speed up and facilitate the purification process, all patient libraries are pooled together into one single tube for cleanup.

This streamlined three-step procedure makes NGS more practical than ever for busy diagnostic laboratories—with hands-on time reduced from days to just minutes, no need for sample splitting, and flexible batch sizes. NGS has never been easier.

→ contents

Equipment requirements

Explore NGS calculators
bit.ly/devyser-ngs-calculators

NGS system
■■■ NGS platforms are differentiated primarily by their underlying sequencing chemistries and detection technologies, which together influence many factors including sequencing read length, error rate, run time, throughput, and depth of coverage.

Compatibility with NGS kits
Before implementing a new NGS-based test, check that the sequencer you will be using is compatible with your preferred diagnostic kit or library construction method. For example, Devyser's NGS kits are designed and optimized for use with Illumina systems (Table 2).

Thermal cycler
A thermal cycler is necessary for PCR amplification and labeling steps during library construction. Since fidelity of DNA amplification is fundamental to generating representative libraries of sufficient complexity, it is important to choose an accurate, robust and reliable system. NGS kit providers should be able to tell you which thermal cyclers have been tested for success with their products.

Depth of coverage
Sequence coverage is a critical parameter that must be optimized for each application. and sequencing system. Devyser provides online coverage calculators to optimize flow-cell usage and achieve the desired coverage for your sequencing run and the lowest cost per sample

→ contents

Additional equipment
The need for additional equipment varies depending on the application and methods used for DNA isolation, quantification and library preparation, but typical requirements include:

- Bench top vortex
- Table top centrifuge
- Strip centrifuge
- Magnetic separator
- Fluorometer

Dedicated single- and multi-channel pipettors are also recommended. For higher throughput applications an automated liquid handling robot or workstation may be useful.

Equipment and materials required for Devyser NGS applications are listed in the Appendix.
In addition, you can use Devyser's NGS checklist to ensure you have everything you need to get started.

Quality control
It is advisable to participate in regular quality control programs to verify the performance of your NGS-assays. Several national and international quality control programs are available for NGS testing.

Talk to an expert
bit.ly/talk-to-a-devyser-expert

→ contents

Planning considerations

System purchase

■■■ Key factors to consider include:

- Sequencing chemistry – some NGS chemistries will require extensive pre-treatment of the library before sequencing, sometimes requiring additional instrumentation
- Total sequencing time – from completed library prep to raw data
- Technical specifications that meet your application and throughput requirements
- Cost – both direct and indirect
- Footprint suitable for designated location in the lab (see Equipment Placement)
- Product support and lifecycle management for associated hardware and software

Financing and alternatives to purchasing

If cost is prohibitive, consider the following alternatives:

- Shared ownership / core facility
- Financing or lease options
- Accessing a system at a local lab or core facility

If you are in need of advice on the best way forward for your lab, contact Devyser. Our field application specialists may be able to help you locate an NGS facility in your area or advise on any of the other options above.

Equipment placement

Clean and dirty work areas

As with any molecular testing procedure, it is good laboratory practice to designate specific "clean" and "dirty" areas for the NGS workflow. Activities should then be organized so that the work proceeds in only one direction: from clean to dirty. Everything up to PCR should be performed in the clean area, while PCR and all post-PCR procedures including sequencing should take place in areas separated from the clean area. Ideally, these workspaces should be located in separate rooms, or at least be physically well separated.

Environmental control

To ensure optimal and consistent performance, the NGS system must be placed in a well-controlled environment. Protection from vibration is particularly important for data quality. If possible, position the system in a low-traffic area to avoid bumps and frequent opening or closing of doors. Other common sources of vibration include HVAC systems, operation of other equipment on the same bench, and even ongoing construction in the vicinity.

To avoid overheating of the sequencer, active cooling of the room and protection from direct sunlight are recommended. In addition, humidity should be monitored and maintained within the manufacturer's recommended range (typically 20-60%).

Validation and legacy testing

As with any diagnostic or laboratory test, standard operating procedures must be established and validated across the entire NGS workflow—from sample preparation through data analysis. Depending on whether NGS equipment and protocols are already established in the lab, sufficient time should be allocated for any IQ/OQ/PQ validation testing required. When complementing or replacing a diagnostic test with an NGS-based method, the new and old procedures are typically run concurrently for a defined

→ contents

period to demonstrate that comparable results can be achieved on a consistent basis. When preparing for this period of legacy testing, check that mechanisms are in place to track each sample through parallel workflows.

Training and assay maintenance

For successful implementation, laboratory staff will require training on all aspects of the NGS workflow. The amount of time required for training can range from days to weeks, depending on the technology being used and whether personnel have prior experience with the relevant molecular techniques and protocols.

To further minimize the training burden and risk of errors, NGS assays and protocols for library preparation should be easy to implement and maintain. In particular, application-specific NGS kits can considerably reduce the amount of training needed for library preparation, which is one of the biggest sources of bottlenecks and error in the NGS workflow. For example, the streamlined workflow of Devyser NGS requires minimal prior experience, other than an understanding of GLP and basic pipetting skills.

In cases where NGS is new to the lab and/or personnel have little prior molecular biology experience, initial training of two dedicated staff members is recommended, so that trainees can support each other through the learning process. Once they have fully mastered the procedures and demonstrated competency, they will be well placed to train additional staff as needed and maintain assay competency in the lab.

"Since the completion of the Human Genome Project, technological improvements and automation have increased speed and lowered costs to the point where individual genes can be sequenced routinely, and some labs can sequence well over 100,000 billion bases per year."

National Human Genome Research Institute

→ contents

Leveraging NGS in routine diagnostics

Contact us
bit.ly/get-in-contact-with-devyser

■■■ Putting the power of NGS to work for routine diagnostic use has never been easier. If you would like to learn more about how your lab can benefit from the latest advances in NGS diagnostics, contact Devyser. Our specialists will be happy to help.

→ contents

Appendix

Devyser NGS kit applications and DNA requirements

Devyser NGS kit	Application	Genes and regions covered	Amount of DNA needed
Devyser BRCA NGS	Breast cancer	Full uniform coverage of BRCA1 and BRCA2, covering all exons and intron/exon junctions	10 ng
Devyser HBOC NGS	Hereditary breast and ovarian cancer	Targeted sequencing of 12 relevant genes: ATM, BARD1, BRIP1, CDH1, CHEK2, NBN, PALB2, PTEN, RAD51C, RAD51D, STK11, TP53 Can be combined with Devyser BRCA	10 ng
Devyser CFTR NGS	Cystic fibrosis	Full CFTR gene sequencing, including all exons and exon/intron junctions, the promoter region and several clinically relevant deep intronic mutations	10 ng
Devyser FH NGS	Familial Hypercholesterolemia	Comprehensive gene panel for Familial Hypercholesterolemia: LDLR, APOB, PCSK9, LDLRAP1, APOE, STAP1	10 ng
Devyser Thalassemia NGS	Thalassemia	Amplicons across the alpha and beta globin gene clusters, including full sequencing of HBA1, HBA2 and HBB genes and robust direct- and coverage-based detection of CNVs	10 ng
Devyser Chimerism	Chimerism measurement and monitoring in transplant patients	Detects down to 0.05% fraction of chimerism	Variable input possible

→ contents

Appendix

Equipment and materials required for Devyser NGS applications

Equipment	Supplier
Sequencing system	Illumina
Thermal cycler	General lab supplier
Table top centrifuge	General lab supplier
Strip centrifuge	General lab supplier
Dynamag-2 magnet	Thermo Fisher
Qubit Fluorometer	Thermo Fisher
Table top vortex	General lab supplier
Reagents and consumable	
Qubit High Sensitivity kit	Thermo Fisher
Denatured, 20 pM PhiX v3	Illumina
PCR reaction tubes/strips or plates for thermal cycling	General lab supplier
Laboratory grade water	General lab supplier
>96% Ethanol	General lab supplier
1 N NaOH	General lab supplier
200 mM HCl-Tris, pH 7.0	General lab supplier

NGS systems compatible with Devyser NGS applications

Platform	Illumina iSeq 100	Illumina MiniSeq	Illumina MiSeq	Illumina NextSeq 550
Maximum reads	4 M	25 M	25 M	400 M
Maximum read length	2x150 bp	2x150 bp	2x300 bp	2x150 bp
Maximum output	1.2 Gb	7.5 Gb	15 Gb	120 Gb
Run time	9.5-19 h	4-24 h	4-55 h	12-30 h

→ contents

Resources

Clinical best practice guidelines

Best practice summarized in a book from 2012
bit.ly/best-practice-in-lab-methods

EMQN Best Practice Guidelines 2015
bit.ly/emqn-best-practice

Guidelines for the clinical management of Thalassemias 2008
bit.ly/guidelines-clinical-management

Standard of care guidelines 2012 for Thalassemias
bit.ly/standard-of-care-guidelines

Eurogenetest general NGS guidelines
bit.ly/euro-gene-test

ACGT - the association for Clinical Genomic Science guideline list including targeted NGS guidelines
bit.ly/acgt-best-practice-guidelines

Guidelines for clinical NGS-based oncology
bit.ly/guidelines-clinical-ngs-based-oncology

Links

Devyser NGS calculators
bit.ly/devyser-ngs-calculators

Talk to an expert
bit.ly/talk-to-a-devyser-expert

Contact us
bit.ly/get-in-contact-with-devyser

EMQN quality programs
bit.ly/emqn-quality-programs

Read more about the product:
bit.ly/devyser-thalassemia-ngs

→ contents