Genetic testing of Thalassemia using NGS

Dvysr®

Challenges in Thalassemia diagnosis

■■■ Thalassemia is typically caused by sequence variants in the HBA1, HBA2 or HBB genes. The common sequence variants include Single Nucleotide Variants (SNVs), smaller insertion and deletions (Indels) as well as large, exon spanning Copy Number Variations (CNVs). A range of different techniques such as GAP-PCR, Sanger sequencing, reverse hybridisation and MLPA is traditionally required to assess all variants.

Testing for both Alpha Thalassemia and Beta Thalassemia can be a complex process. Workflows are laboratory-specific and often require the use of several different techniques to obtain a result. Relying on a patchwork of methods presents challenges such as:

- Long turn-around times to get an overview of both alpha and beta thalassemia sequence variants in a patient
- Resource-intensive and costly processes to validate, maintain and train operators on all assay types
- Risk that sequence variants remain undetected if the workflow is terminated when a first sequence variant is found, or a method is used where only sequence variant-specific detection is possible
- Risk for sample contamination and mix-up when handling multiple tubes and protocols

One assay for all Thalassemia needs

■■■ The Devyser Thalassemia kit is a single, one-tube, NGS assay that detects all sequence variants in HBA1, HBA2 and HBB in a single run, eliminating the need for additional workflows. The assay detects SNVs, Indels and uses two independent methods in parallel to detect CNVs.

Simultaneous comprehensive analysis of the HBA and HBB gene clusters offers major advantages. Devyser's fast and simple NGS workflow replaces complex multi-step protocols and eliminates the need for maintaining multiple Thalassemia assays in your lab. The simple procedure improves workflows in any type of lab, whether you are performing advanced second level genetic testing, or large-scale sequence variant screening. The simple workflow significantly reduces the risk for contamination and sample mix-up.

Devyser also provides an online tool to help you plan the NGS run for optimal flowcell input to help you reduce costs and save time.

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

Prof. Dr. Veysel Sabri Hancer
Istinye University Genetic Diseases Diagnostic Centre
Istanbul, Turkey

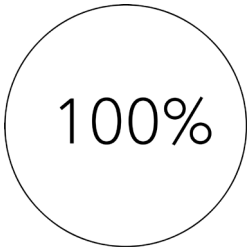
Detect all sequence variants

■■■ If a genetic testing workflow is terminated when a first sequence variant is found, or a method is used where only sequence variant-specific detection is possible, there is a risk that additional sequence variants remain undetected.

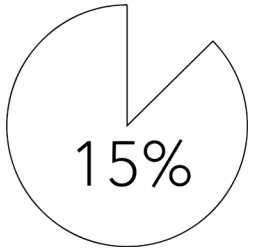
A recent study shows that the Devyser Thalassemia NGS assay detects sequence variants that were not discovered with traditional Thalassemia testing. Devyser's kit was used to analyse 125 samples previously characterised to carry pathogenic sequence variants in the globin genes using traditional genetic testing methods and workflows.

- All previously detected pathogenic SNVs, Indels and CNVs in the globin genes were confirmed using Devyser Thalassemia.
- In 15% of samples, additional pathogenic sequence variants were found. The majority of these were found in a different globin gene. These sequence variants had previously not been identified since the workflow had been terminated as soon as a pathogenic sequence variant was found in the first gene cluster investigated.

125 characterised clinical samples analysed with Devyser Thalassemia



100% of previously characterised pathogenic



15% of samples contained additional, previously undetected pathogenic variants.

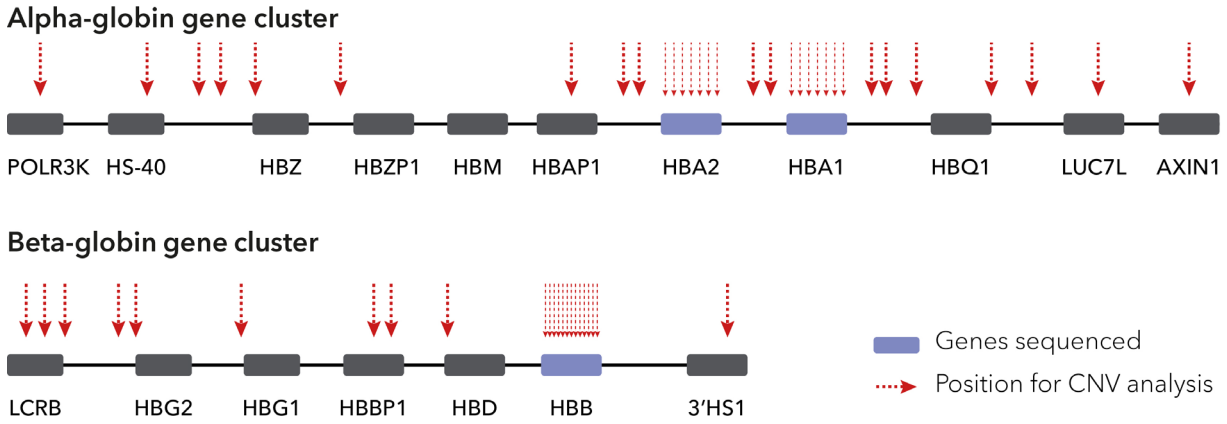
Detect all sequence variants

Genes and regions covered

■■■ Large structural deletions are analysed over the entire alpha and beta globin gene clusters. The HBA1, HBA2 and HBB genes have an extra high coverage density to allow precise deletion mapping.

Genes colored in purple are fully sequenced to allow comprehensive detection of Indels and point sequence variants in the globin genes. Vertical arrows illustrate positions where the presence of CNVs (i.e. large structural deletions) are investigated.

Schematic overview of the alpha and beta globin gene clusters



Powerful CNV detection

■■■ NGS detection of CNVs is typically achieved by comparing the sequence coverage of regions between or within samples. This coverage-based CNV method can be challenging as it requires an even and reproducible sequence coverage of the target regions; normalisation within and between samples, as well as the use of control samples.

Devyser's unique PCR chemistry allows detailed coverage-based CNV analysis thanks to its ability to amplify long stretches of overlapping amplicons with an even, and reproducible, sequence coverage. This is a powerful method to detect both pre-characterized CNVs as well as those previously uncharacterized. Data analysis is performed with a tailored bioinformatic software pipeline using algorithms for reliable detection and mapping of CNVs.

The detection of CNVs is further improved through the use of PCR primers aligned to both ends of 18 CNVs with high prevalence. This is referred to as Direct Detection of CNVs, and is a reliable and robust method to detect CNVs with well characterised breakpoints. The software pipeline uses the combined results of the two CNV methods to map and confirm findings.

Alpha globin deletions and mode of detection

7 direct CNV detections of larger deletions

Deletion	Direct Detection of CNV	Coverage based CNV detection
--SEA	●	●
--FIL	●	●
--THAI	●	●
-(α)20.5	●	●
--MED	●	●
-(α)21.9	●	●
-(α)27.6	●	●
-(α)3.7		●
-(α)4.2		●
HS-40		●
Other deletions		●

Beta globin deletions and mode of detection

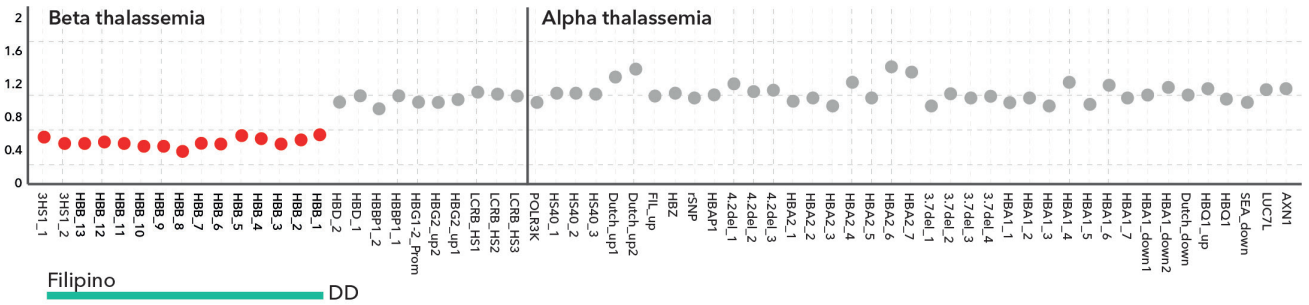
11 direct CNV detections of larger deletions

Deletion	Direct Detection of CNV	Coverage based CNV detection
Chinese	●	●
Filipino	●	●
Yunnanese	●	●
Taiwanese	●	●
SEA-HPFH	●	●
δβ-Sicilia	●	●
Hb-Lepore Boston	●	●
Hb-Lepore Baltimore	●	●
Hb-Lepore Hollandia	●	●
290bp-del	●	●
B619-del	●	●
Other deletions		●

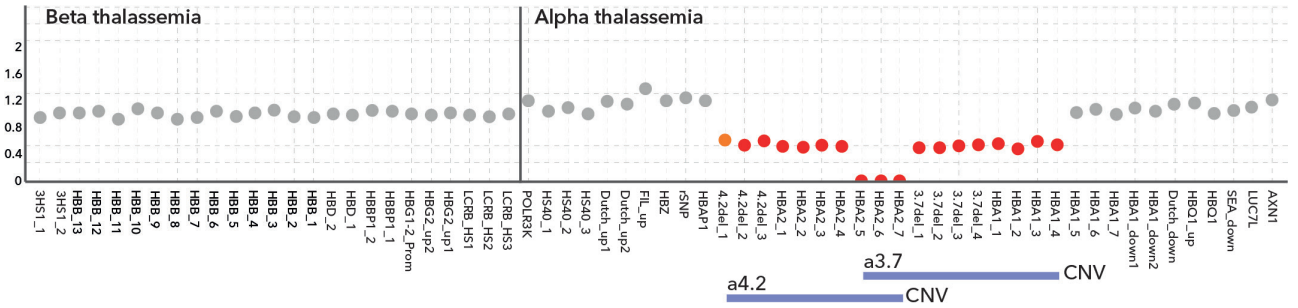
Data analysis

■■■ A tailored bioinformatic software pipeline enables data analysis and assists in the clinical interpretation of testing results obtained using Devyser Thalassemia. SNVs, Indels and CNVs are rapidly analysed and intuitively displayed.

A sample with the Filipino deletion in the beta globin genes detected by Direct Detection of CNVs.



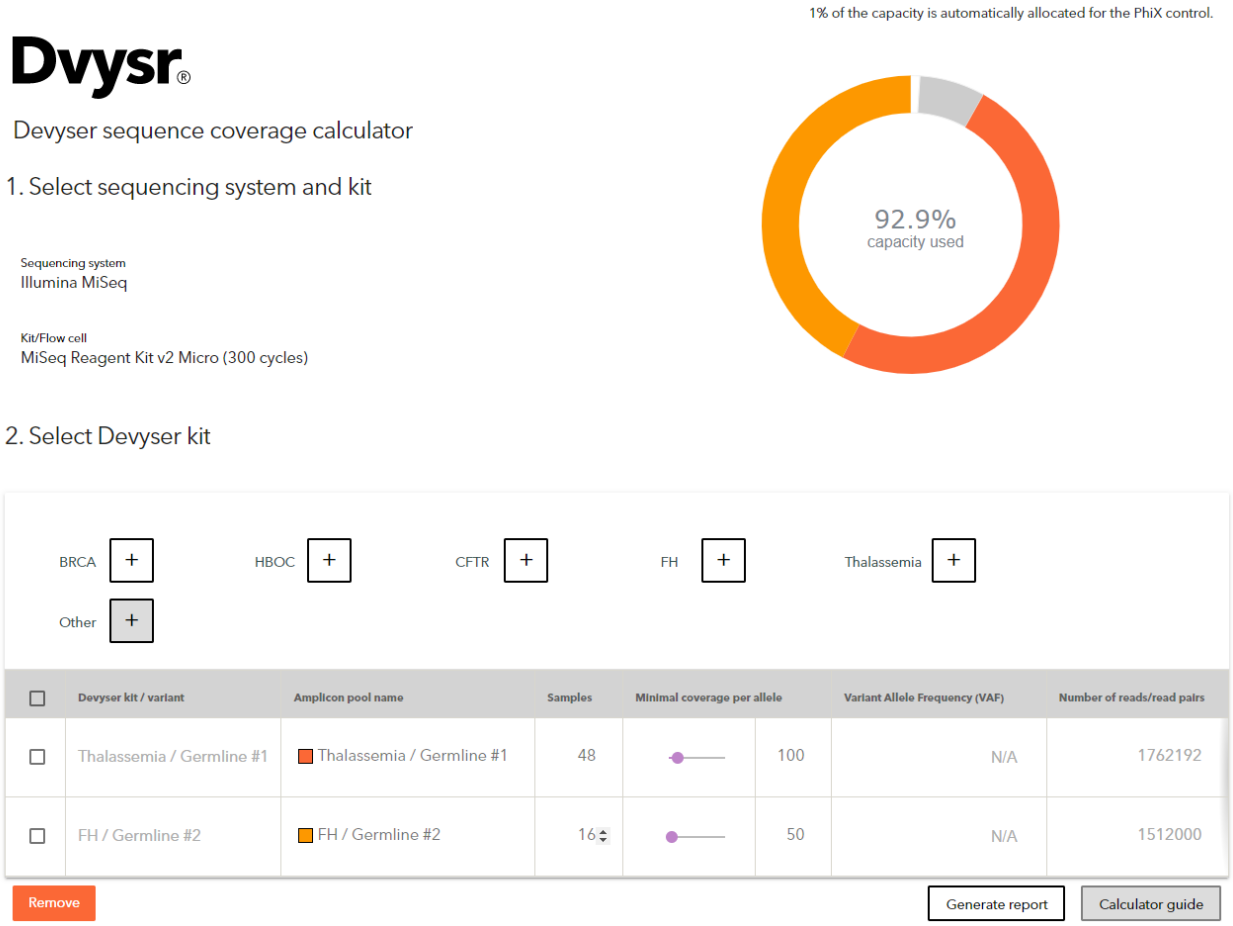
A sample with heterozygous compound deletions (a3.7 and a4.2) in the alpha globin genes, detected by coverage-based CNV analysis.



Sequence planning

■■■ Devyser offers an online Sequence Coverage Calculator to help plan your NGS run for optimal results.
Simply select your sequencing instrument and the flow cell you would like to use. The tool will then suggest the optimal number of samples to be pooled for maximum flow cell use and cost-efficiency.

Different Devyser NGS kits can be used and combined for sequencing. If different libraries are combined, the Sequence Coverage Calculator can calculate the optimal amounts of the different NGS libraries that should be sequenced together.



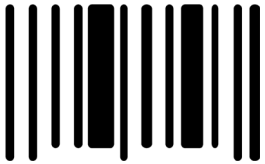
Fast and simple NGS workflow

■■■ Devyser's NGS kits offer a single tube library protocol with indexes that are delivered pre-dispensed in strip or plate format. This workflow minimises hands-on time and significantly reduces the risk for sample mix up and contamination. All patient samples are pooled to a single tube before clean-up.

Target amplification

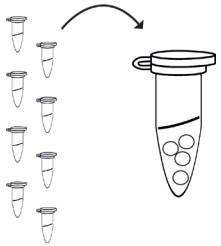
All target sequences are amplified using one single tube per patient sample.

Index addition



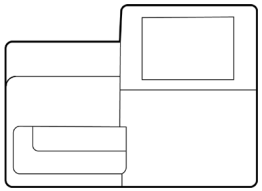
Patient specific molecular indexes are added to all samples.

Pooling and cleanup



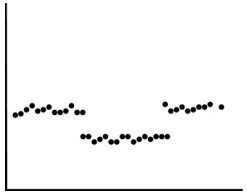
Combine and purify up to 96 samples simultaneously in a single tube.

NGS



Patient samples are sequenced using Next Generation Sequencing.

Data analysis



Data analysis and results reporting using a tailored Thalassemia analysis pipeline.

Assay details

Number of mixes	1
Optimal input DNA	10 ng
Sequencer	Illumina iSeq, MiSeq and MiniSeq
Coverage uniformity (>0.2x mean coverage)	>99.9 %
Size of amplified region	9.4 kb
Total hands-on time	<45 min.
Total library preparation time	5 h.

From DNA to sequencing in under 5 hours with less than 45 minutes hands-on time.

About Thalassemia

■■■ Causes

Thalassemias are haemoglobinopathies characterised by an abnormal haemoglobin production that can lead to anaemia and destruction of red blood cells. Alpha and beta thalassemia, the two main types of thalassemia, are caused by inadequate production of the α and/or ß globin molecule.

Symptoms

Symptoms of thalassemia vary from mild, to severe and even fatal. In 1990 more than 36,000 deaths were reported due to thalassemia. This has decreased to 16,800 in 2015 primarily due to national screening programmes and family planning. Approximately 5% of the global population has a variant in the genes coding for the alpha or beta haemoglobin molecules. Of these, 34% have symptoms.

Prevalence

Alpha thalassemia is most common in sub-tropical and tropical areas. In certain ethnic groups, the prevalence can be as high as 30%, with up to 90% as carriers. Beta thalassaemia is particularly revalent among the Mediterranean, African and South Asian population. It is hypothesised that alpha and beta thalassemia are more prevalent in malaria exposed regions due to improved disease protection.

Short facts - Devyser Thalssemia for NGS

- Single-tube NGS assay for simultaneous comprehensive analysis of the HBA and the HBB gene clusters
- Full gene sequencing of HBA1, HBA2 and HBB genes enables detection of all SNVs
- Robust CNV detection with two combined strategies for CNV detection:
  · Direct detection of 18 CNVs
  · Coverage based detection of CNVs in both the HBA and the HBB gene clusters
- Built in rapid sample mix-up control through sex chromosome markers

Read more about the product:

bit.ly/more-about-thalassemias
bit.ly/more-about-devyser-thalassemia
bit.ly/chimerism-knowledge-hub

Article numbers
Devyser Thalassemia
8-A106-24 (24 tests)
8-A106-48 (48 tests)
8-A106-96 (96 tests)

Accessories
Devyser Library Clean
8-A204

Devyser Index Plate A
8-A200

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