Sanger Sequencing in Diagnostic Labs

The sequencing of single or multiple genes is often the first step in the genetic diagnosis of a disease and informs subsequent treatment and care. The development of the Sanger sequencing method in 1977 allowed labs to sequence DNA fragments with automated base calls for the first time. While continual developments have led to innovative technologies such as Next Generation Sequencing (NGS), Sanger sequencing is still considered the gold standard technique for some clinical scenarios, for example, 16S sequencing, human leukocyte antigen (HLA) typing, microsatellite instability (MSI), tandem repeats, and rare variant confirmation.[1,2]

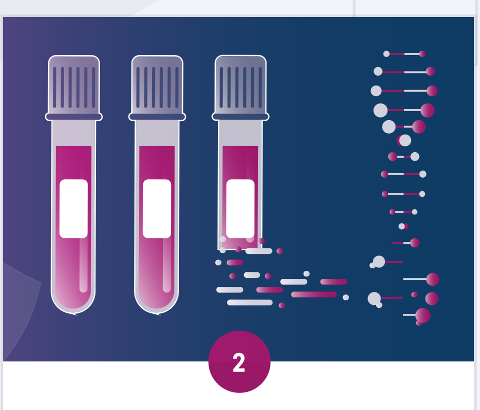
This infographic will explore the benefits of using Sanger sequencing in diagnostic laboratories.

Sequencing in a Diagnostic Laboratory[3]

1

DNA or RNA is extracted from the diagnostic sample (the latter is then converted to cDNA).

Correct sample handling and extraction are critical for producing the high-quality DNA template needed for sequence analysis.



2

The extracted sample is sequenced.

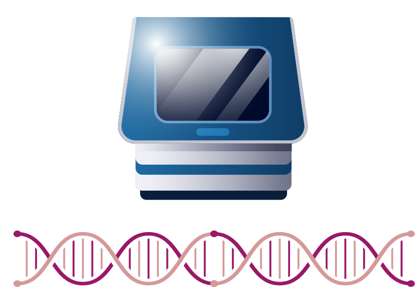
3

Sequencing data is analysed by a trained scientist and any unusual laboratory findings are verified.

Sanger vs. NGS sequencing for Clinical Decisions

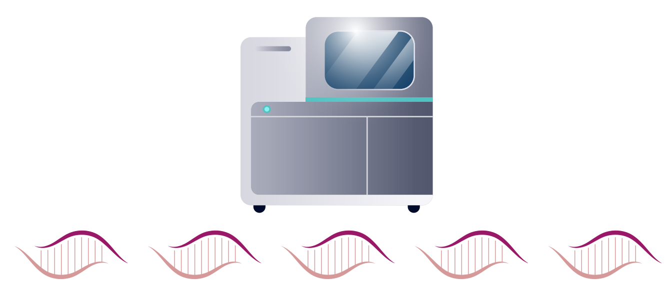
Genetic/genome sequencing is critical for the management of diseases with a genetic component and is increasingly becoming part of clinical interventions. Sanger sequencing and NGS are both highly valuable and complementary tools for diagnostic laboratories. However, there are some distinct differences between the two methods, which include:

Sanger sequencing



The sequencing of single genes or regions (up to 1,000 base pairs)

NGS sequencing



The simultaneous sequencing of thousands of genomic variants (either concurrently identifying many individual changes in one sample or comparing many samples at once)

APPLICATIONS

Sanger sequencing	NGS sequencing
Pathogen detection	Microbiome analysis
Validation of PCR results	Complex genetic aetiology
Monogenic diseases	Pathogen subtyping in critical outbreak situations
Short tandem repeat analysis	Sequencing the entire genome/exome to find novel variants

Comparison of Sequencing Techniques

Sanger sequencing		NGS sequencing
Can only sequence a single fragment at a time Can view the trace directly for challenging templates and interpretation by user	DATA	Can sequence millions of fragments in parallel per run Large data set which requires specialist training and resources to interpret
Fast for low number of targets and therefore a fast turnaround time	TIME	Slower overall therefore longer turnaround time, but can give data on multiple targets at once
Cost-effective for low number of targets such as in the screening and diagnosis of monogenic disorders NHS single-gene Sanger sequencing reimbursement rate was £138 in one study[4]	COST	Cost-effective for higher sample throughput and for sequencing across a large number of genes, as is necessary for the screening and diagnosis of diseases with complex genetic aetiology such as cancers In one study, the NHS single gene NGS diagnostic cost £339 per patient[4]

NGS is becoming more accessible to clinical labs. However, approval and validation of NGS-based laboratory-developed tests (LDTs) by administrations, such as the U.S. Food and Drug Administration (FDA), is a difficult process requiring high expertise and investment. In contrast, Sanger sequencing is still a valuable asset as it has a short turnaround time and low cost making its validation uncomplicated and cost-effective.

Introducing QuantumSeq™ Sanger Sequencing Reagents

There are a variety of Sanger sequencing reagents available on the market therefore it is critical to know which reagents produce the best results for the best price. QuantumSeq™ Sanger Sequencing reagents are cost-effective, produce high-quality data, and can be used as direct substitutes for other products from leading competitors.

Key Features

High-quality results

Generates uniform peak heights with extremely low background and long sequence reads with high confidence scores.

Compatible

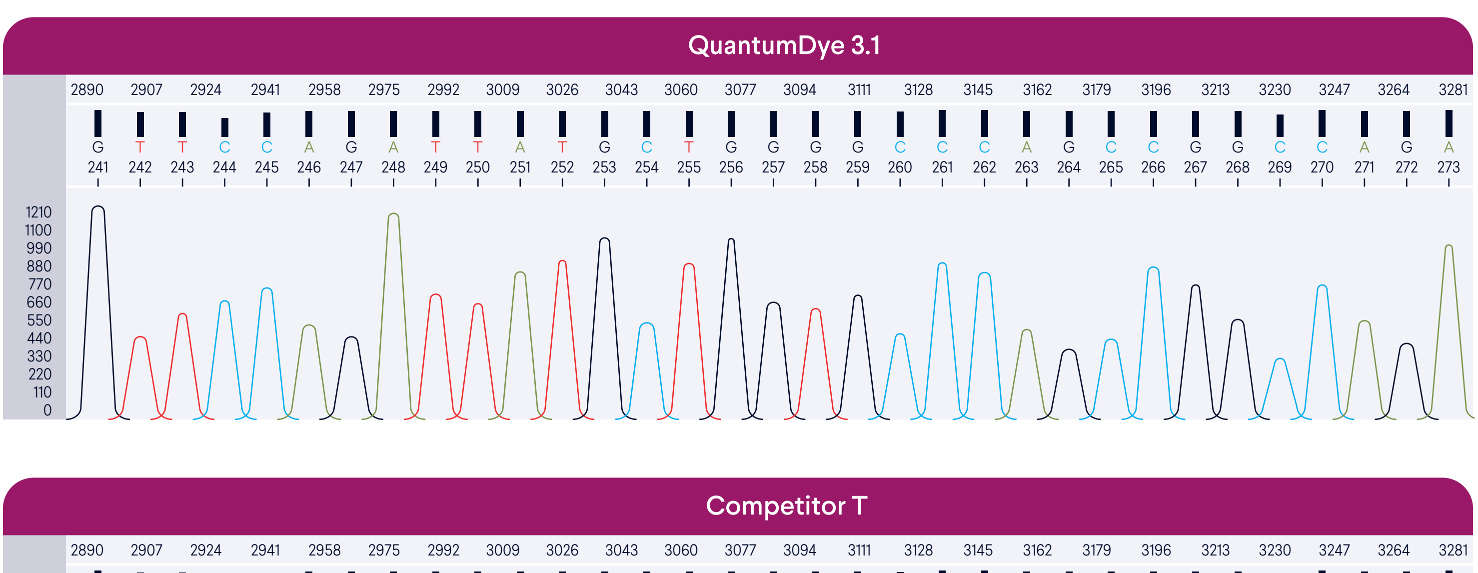
Direct, drop-in substitute for BigDye® Terminator v3.1, with no changes in protocol, dilution, workflow, calibration, or settings using DyeSet Z.

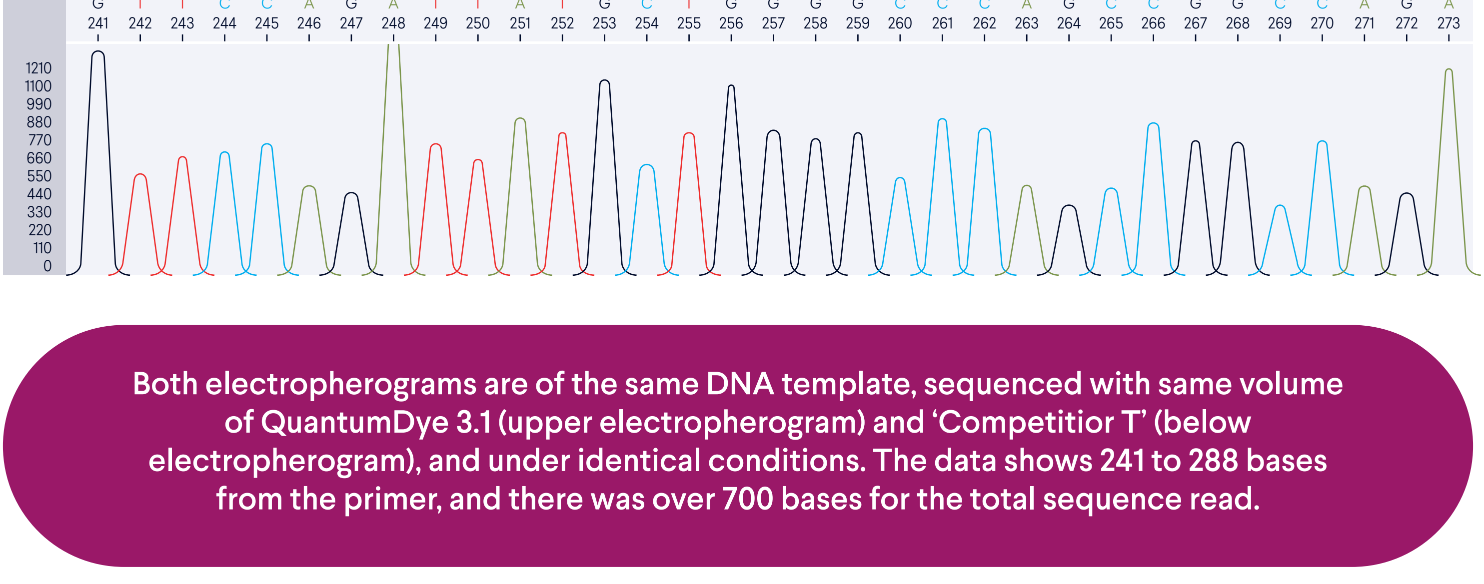
Cost-effective

Able to provide accurate results with reduced sequencing costs for low target number compared to NGS.

Challenging Competitors

QuantumSeq™ Sequencing provides the same high performance but at a greater value compared to the market leader. The kit provides identical and uniform peak heights/intensities and confidence scores, along with high-resolution cycle sequencing with extremely low background noise.





Both electropherograms are of the same DNA template, sequenced with same volume of QuantumDye 3.1 (upper electropherogram) and 'Competitior T' (below electropherogram), and under identical conditions. The data shows 241 to 288 bases from the primer, and there was over 700 bases for the total sequence read.

References

1. Hartman P, Beckman K, Silverstein K et al. Next generation sequencing for clinical diagnostics: Five year experience of an academic laboratory. *Mol Genet Metab Rep.* 2019;19:100464. doi:10.1016/j.ymgmr.2019.100464

2. Gomes A, Korf B. Genetic Testing Techniques. *Pediatric Cancer Genetics*. 2018:47-64. doi:10.1016/b978-0-323-48555-5.00005-3

3. Crossley B, Bai J, Glaser A et al. Guidelines for Sanger sequencing and molecular assay monitoring. *Journal of Veterinary Diagnostic Investigation.* 2020;32(6):767-775. doi:10.1177/1040638720905833

4. Hamblin A, Wordsworth S, Fermont J et al. Clinical applicability and cost of a 46-gene panel for genomic analysis of solid tumours: Retrospective validation and prospective audit in the UK National Health Service. PLoS Med. 2017;14(2):e1002230. doi:10.1371/journal.pmed.1002230