Low-Cost Sanger Sequencing

Gerbera Sequencing Kit v3.1

Sanger sequencing has been widely used and popular method for decades. This method improved over the years, mainly due to automation, and was the basis for sequencing the first human genome in 2000. In less than two decades, however, Next-Generation Sequencing (NGS) has overtaken Sanger sequencing due to its high throughput and much lower cost per base. Yet Sanger sequencing is definitely not going away. For many users and projects, it is the first and obvious method of choice and remains considered the gold standard sequencing method for validating the sequence of specific genes, including those already sequenced through NGS.

Same as the method itself, Sanger chemistries have also changed over time to provide longer and more robust reads. But for years now the market has been dominated by BigDye® kits developed at Applied Biosystems corporation, namely by the BigDye® Terminator kit v3.1. In effort to develop a cost-effective alternative to this sequencing kit, we are proudly announcing our new Gerbera Sequencing Kit v3.1.

Immediate Integration

Dyes in the new Gerbera Sequencing Kit v3.1 have same spectral characteristics as those in the BigDye® Terminator kit v3.1 and thus, no instrument recalibration is required for data analysis. Also, the kit protocol and whole workflow have been designed to use exactly same conditions as for the original BDT kit. Therefore, researchers can easily integrate our new Gerbera Sequencing Kit v3.1 into their workflow.

Guaranteed Performance

Gerbera Sequencing Kit v3.1 is quality tested for correct formulation and then for consistent, reliable performance. Namely, the following parameters are evaluated – QV20+ Report, Contiguous Read Length, Sample Score, Base Calling Accuracy and Fluorescence Background. Additionally, our expert support team is readily available to provide whatever assistance you may require.

GERBERA SEQUENCING KIT v3.1

Specifications

Gerbera Sequencing Kit v3.1 includes the sequencing chemistry itself (provided as a ready-to-use single tube mix) and 5× Sequencing Buffer that enables diluting the kit to save even more on cost per sample. Reagents are optimized for use with all commonly used Sanger genetic analyzers available.

Sequencing Kit Version	Gerbera Sequencing Kit v3.1	BigDye® Terminator kit v3.1
Instrument compatibility	Identical	
Applications		
Kit Protocol		
Spectral calibration *		
5× Dilution buffer **	Provided	Provided

* There is no need to perform additional spectral calibration when implementing the Gerbera Sequencing Kit v3.1 into your workflow. Simply run your samples on your genetic analyzer as if they were prepared by the BDT kit. Optimization of injection parameters may be necessary.

* * The buffers are not guaranteed to be identical. We recommend using the original 5× Dilution buffer.

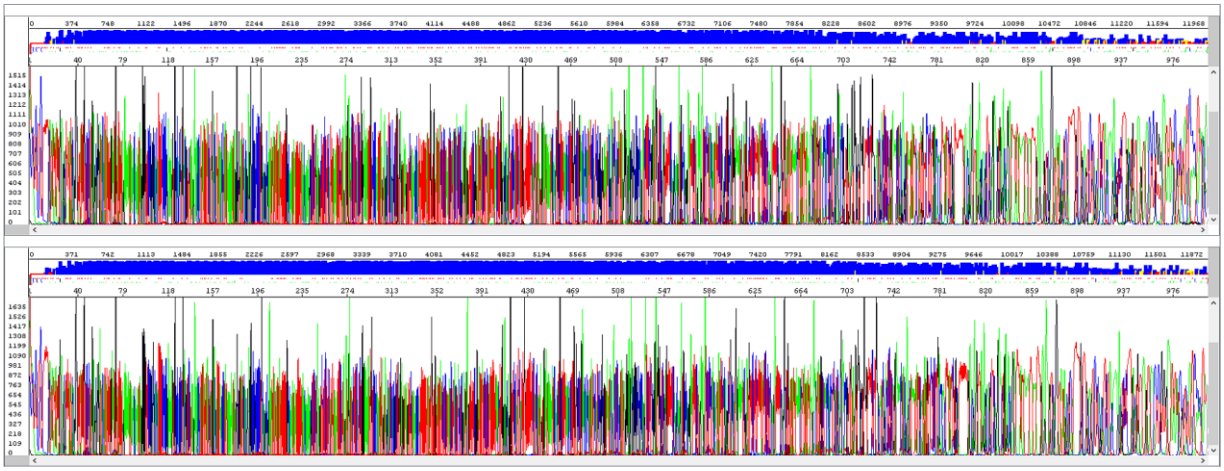
Comparison of Kits

We internally tested various template types at optimal/ suboptimal concentrations, plasmids and PCR products, including templates containing inhibitors or having GC-rich stretches, repeats or hairpins.

Data for three representative samples are presented. Upper panel always shows a sample sequenced using the Gerbera Sequencing Kit v3.1. Lower panel shows the same sample run under identical conditions with the BigDye® Terminator kit v3.1. All samples were run using 50 cm capillary array and POP-7™ polymer on 3130xl Genetic Analyzer. Data were analyzed automatically by standard basecallers with no human intervention.

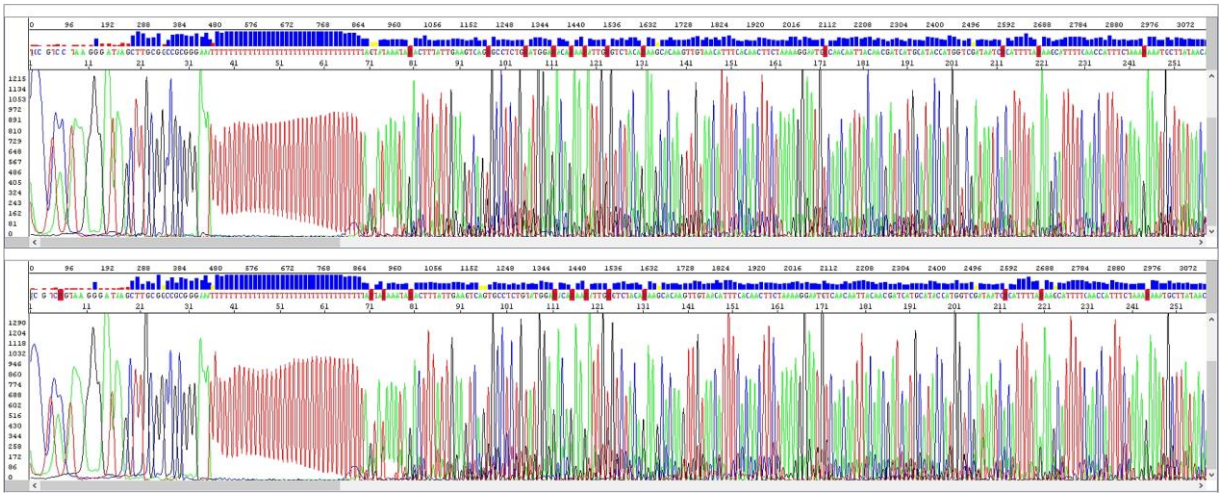
Example 1 *Here the Gerbera Sequencing kit v3.1 performs slightly better, as demonstrated by the following metrics:*

Sequencing Kit Version	Gerbera Sequencing Kit v3.1	BigDye® Terminator kit v3.1
QV20+	895	873
Contiguous Read Length	993	987
Sample Score	30	28
Average Signal-to-Noise ratio	230	296



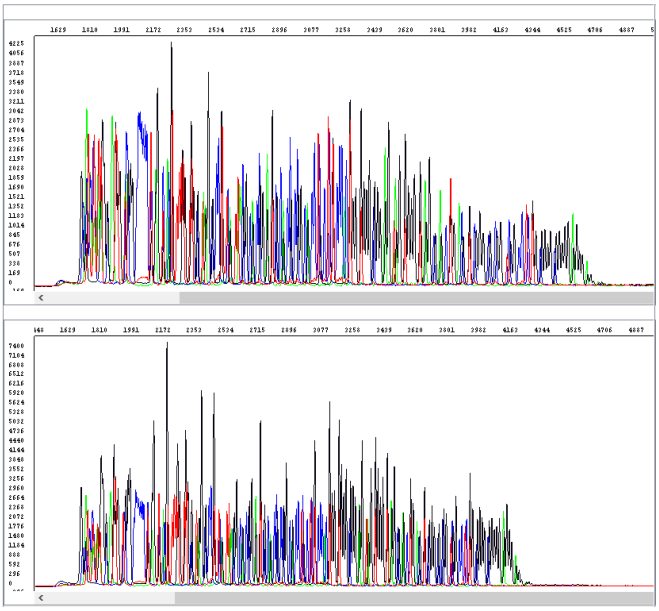
Example 2 *Here the BigDye® Terminator kit v3.1 performs slightly better than the Gerbera Sequencing Kit v3.1. Notice, however, equal trace quality after the polyT stretch. Only first approx. 250 bases are pictured for better visualization of similarity.*

Sequencing Kit Version	Gerbera Sequencing Kit v3.1	BigDye® Terminator kit v3.1
QV20+	905	932
Contiguous Read Length	948	980
Sample Score	45	46
Average Signal-to-Noise ratio	379	458



Note: In examples 1 and 2, all results meet manufacturer specifications for the instrument configuration used.

Example 3 *Both kits perform similarly also with difficult sample types. This is demonstrated by a sample having a polyG stretch causing the sudden signal drop. Raw data shown.*



The Added Value

As demonstrated by all examples, the differences between kits are minor and neglectable. This conclusion can be made for all the samples we evaluated (data not shown). Depending on the particular sample, results were slightly better either by using one or the other kit but we observed no strong preference for any of them and we were not able to recognize which kit was used when comparing samples side by side.

We conclude that the Gerbera Sequencing Kit v3.1 performs equally well as the BDT kit regardless of the sample type. Individual modifications of the recommended protocol had a comparable effect on the results obtained by both kits tested.

The Gerbera Sequencing Kit v3.1 is therefore an excellent solution for all laboratories performing Sanger sequencing. Its implementation is immediate and it brings the added value of significantly lowered cost per sample.

Ordering Information

- Gerbera Sequencing Kit v3.1 is available in 125, 250, 500 and 1000 reaction packages.
- Each kit contains 5× Sequencing Buffer.
- The kit expiry date is 12 months since the date of manufacture when stored at -20°C.
- Feel free to inquire about lot availability.

Request your free sample or mail your orders to info@seqme.eu!