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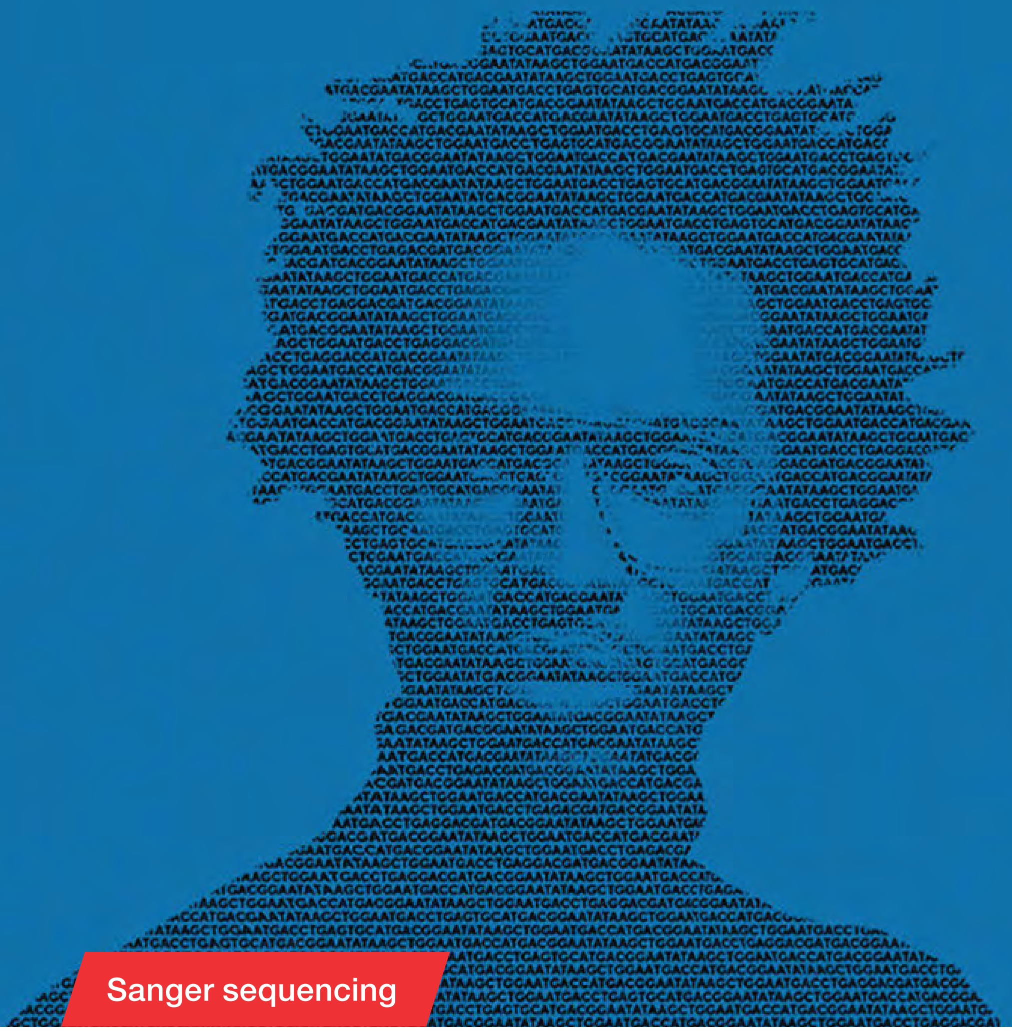


Sanger sequencing

Comparison of BigDye™ Terminator v3.1 Cycle Sequencing Kit to alternative product

Sequencing challenging templates

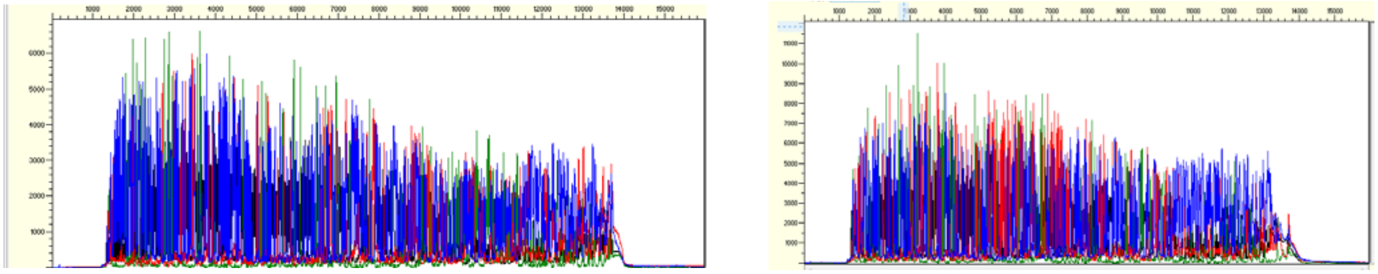
applied biosystems

Applied Biosystems™ genetic analysis systems and consumables are widely used and trusted for Sanger sequencing. The BigDye Terminator v3.1 Cycle Sequencing Kit is expertly designed to meet the demands of a vast majority of applications and challenging templates. BigDye sequencing reagents are rigorously tested for quality. This commitment to quality has helped Applied BioSystems BigDye Terminator product line remain an industry leader for over 25 years with over 15,000 publication references.

In a study, we compared our Applied Biosystems BigDye Terminator v3.1 Cycle Sequencing Kit with an alternative product over a range of challenging templates such as high GC rich content and homopolymers.

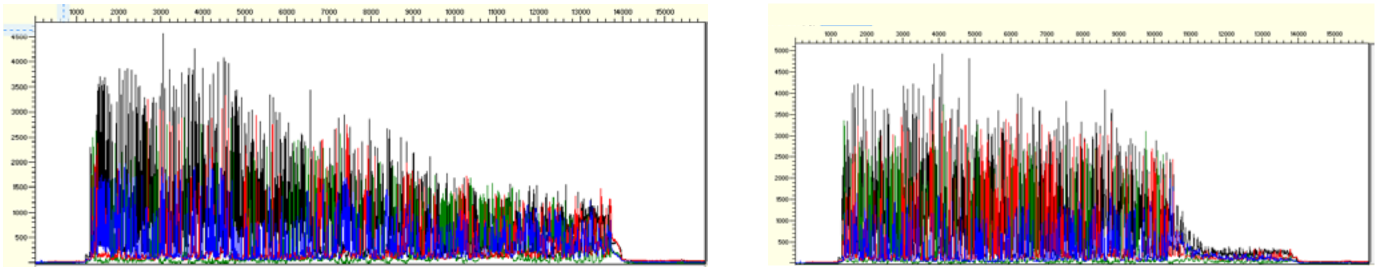
High GC rich content

When running BigDye Terminator v3.1 kit and Product M kit with a high GC rich template, BigDye terminator v3.1 kit resulted in higher signal for both the forward and reverse reads from 5' to 3' as shown in Figures 1A and 1B, whereas Product M kit had a drastic decline in signal as shown in Figures 1C and 1D. BigDye Terminator v3.1 kit outperforms Product M when sequencing templates of high GC content with higher overall signal and longer contiguous read length (CRL).



A forward read 0.5µL BigDye kit

B reverse read 0.5µL BigDye kit



C forward read 0.5µL Product M kit

D reverse read 0.5µL Product M kit

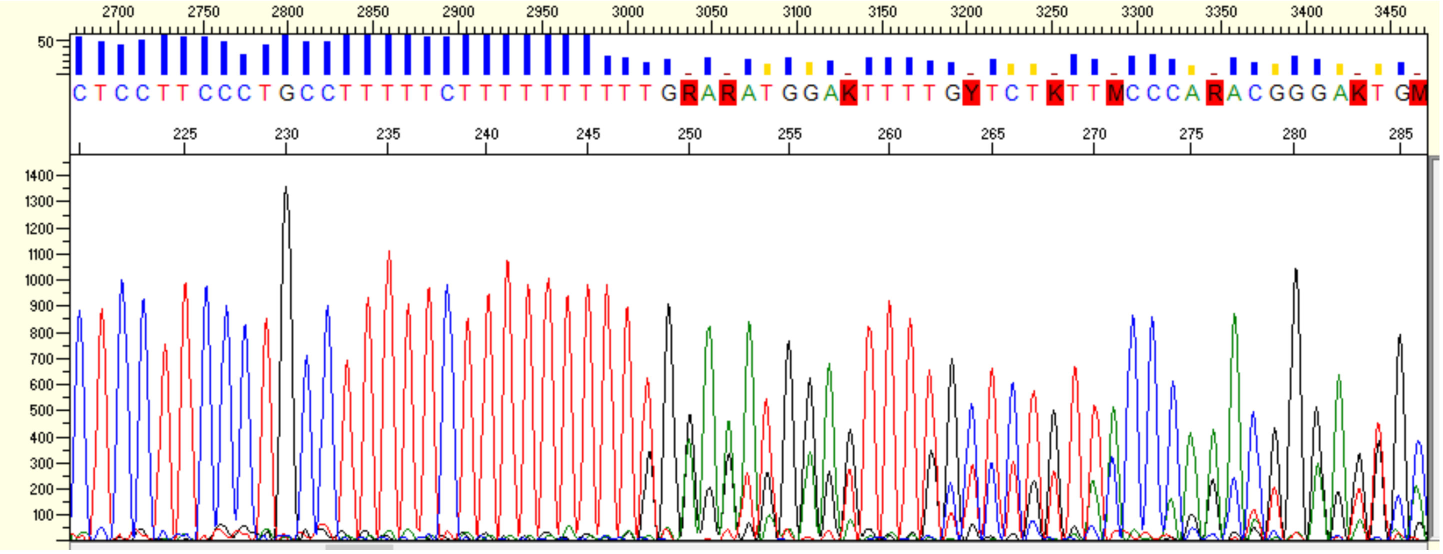
Figure 1. Raw data are shown for reactions made with 0.5µL of each Cycle Sequencing kit for both forward and reverse sequencing reads of a rich GC rich template. (A,B) BigDye kit has high 5' to 3' signal throughput the sequences. (C,D) Shows a steady to drastic decline in signal intensity toward the 3' end of the reads using alternative Product M kit.

Homopolymer regions

Another challenging template to sequence are homopolymer regions, which are regions where there is a stretch of the same nucleotide and is a common source of sequencing errors. Homopolymer regions can be difficult to sequence due to the dynamics of the enzymes used in the polymerase reactions causing the number of identical nucleotides in such regions not being accurately accounted for by the polymerase, referred to as polymerase slippage. The polymerase slippage leads to the loss of a base in the sequence called an N-1 error.

BigDye Terminator v3.1 and Product M were used to sequence a homopolymer region of a string of thymine (T). The results in Figure 2 show that BigDye Terminator v3.1 chemistry has less polymerase slippage than Product M and can sequence through homopolymer regions resulting in better quality data that can be analyzed using secondary analysis software such as KB and Smart Deep Basecaller. Whereas Product M chemistry resulted in loss of base calling after the homopolymer repeat due to polymerase slippage and poor quality data that cannot be analyzed.

A forward read 0.5µL BigDye Terminator v3.1 Kit



B forward read 0.5µL Product M Kit

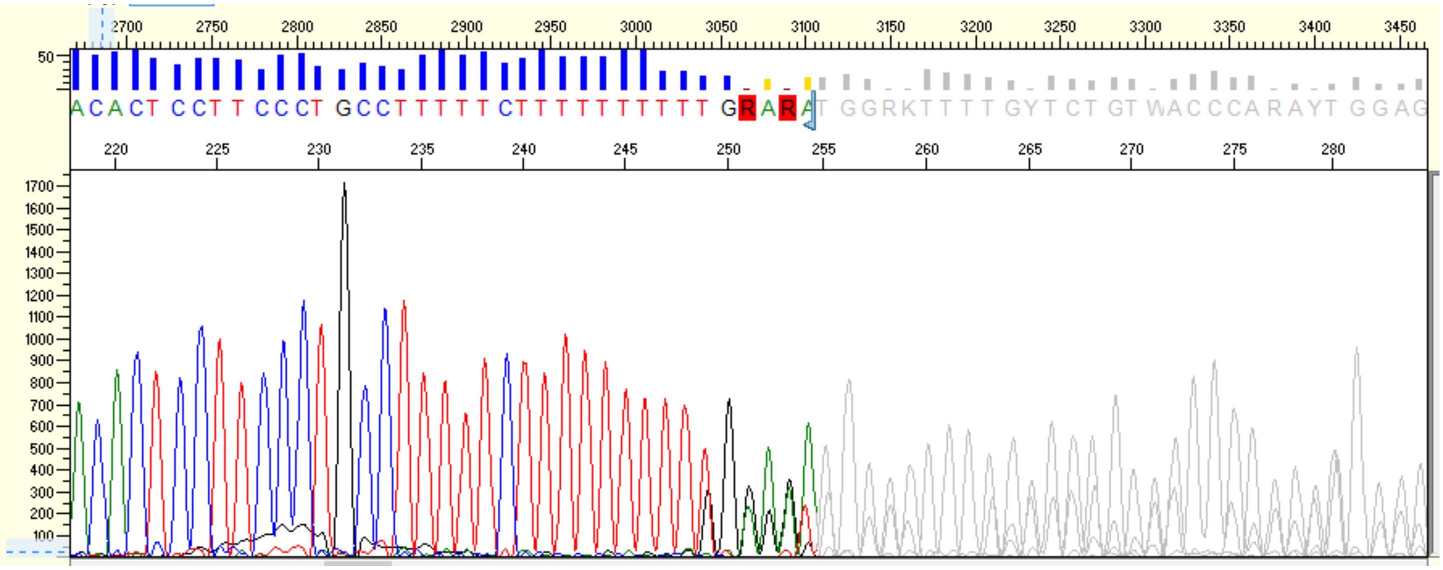


Figure 2. Raw signal plot are shown for reactions made with 0.5µL of each Cycle Sequencing kit for forward sequencing reads of a thymine (T) homopolymer repeat region. (A) BigDye Terminator v3.1 chemistry shows base calling after homopolymer repeat with a more robust polymerase performance whereas (B) Product M chemistry results in loss of base calling after the homopolymer repeat due to polymerase slippage.

Summary

BigDye Terminator v3.1 produces longer CRLs than Product M, resulting in better performance with challenging sample types. BigDye Terminator v3.1 signal intensity is consistently higher than Product M across a broad range of template types, including higher overall signal intensity across the full-length template with challenging high GC content and the ability to better handle homopolymer stretches accurately resulting in more usable data for further analysis. BigDye Terminator v3.1 provides accurate and useable data on a broad range of sample types.

Performance Feature	Thermo Fisher Scientific® BigDye Terminator	Product M
Long Templates	*****	***
Raw Signal Intensity	*****	*
5’-3’ signal balance	*****	**
Contingous Read Length	*****	***
Homopolymer Reads	*****	**

Ordering information

Product	Quantity	Cat. No.
BigDye Terminator v3.1 Cycle Sequencing Kit	100 reactions	4337455
BigDye Terminator v1.1 Cycle Sequencing Kit	100 reactions	4337450
BigDye Direct Cycle Sequencing Kit	100 reactions	4458687
Sanger Sequencing Kit	200 reactions	A38073
Sequencing Analysis Software v 8.0	1 license	A38938
Smart Deep Basecaller Software	1-year license	A53212
SeqScape Software v4.1	Initial license	A53548
Variant Reporter Software v3.1	Initial license	A53552

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