SupreDye™ Cycle Sequencing Kit for Sanger Sequencing

ABSTRACT

AdvancedSeq SupreDye is a cost-effective, high-quality dye chemistry for Sanger sequencing, which decreases the operation cost and provides a powerful alternative for the BigDye chemistry. Here we demonstrate that the AdvancedSeq SupreDye Cycle Sequencing Kit generates higher-quality sequencing results when compared to the sequencing kit from Vendor T using regular (average GC and no apparent secondary structure) and difficult (with secondary structures) templates. Designed for high GC-content templates, the SupreDye dGTP Cycle Sequencing Kit provides significantly better performance compared to SupreDye Cycle Sequencing Kit using a high-GC content templates.

INTRODUCTION

Automated Sanger sequencing using the BigDye Terminator Cycle Sequencing Kit has expedited molecular research by facilitating the identification and confirmation of DNA/gene mutations and in de novo DNA sequencing and resequencing, and has made significant contribution to the Human Genome Project. The linear cycle sequencing method uses dideoxy chain termination to produce a nest of fluorescent extension products with only one nucleotide difference. The extension products are then resolved using capillary electrophoresis and the fluorescent end-labeled ddNTPs are detected by a laser. Base calling is made on the software to compose the DNA sequence based on the template.

However, the mature Sanger DNA sequencing service has been facing competitive price challenges although it is still the gold standard and the most commonly used sequencing technique. It is hard to maintain superior sequencing service quality for the scientific community without properly managing the reagent cost. Cost issues have become one of the important considerations for service providers.

We have developed SupreDye Cycle Sequencing Kits as an alternative to the BigDye Terminator Cycle Sequencing Kit. In this application note, we demonstrate that

(1) Comparable quality of sequencing data is generated from SupreDye chemistry and the chemistry of another brand from Vendor T (Dye from Vendor T) using regular templates (no secondary structure or high GC content);

(2) Better performance is observed for SupreDye chemistry compared to Dye from Vendor T when a difficult template with secondary structure was used;

(3) The specially designed SupreDye dGTP Cycle Sequencing Kit performs better than the regular SupreDye Cycling Sequencing Kit when the template contained high GC content.

MATERIALS AND METHODS

Sequencing Templates: The three templates used in this application note are listed in Table 1. All templates were plasmids obtained from private resources.

Table 1. Templates used in this application note.

Templates	Features
Regular Template	Regular GC content (whole region between 30 and 80%)
Difficult Template	Contains secondary structure
High GC Template	High GC-content (in over 80% of theregions)

Sequencing Reagents for Preparation of Samples for Sequencing: Advanced Seq sequencing reagents used are listed in Table 2. They were compared with Sanger sequencing reagent from Thermo Fisher Scientific for reference.

Table 2. AdvancedSeq Sequencing reagents used for this application note.

	AdvancedSeq	Thermo Fisher Scientific
Sequencing Reaction Kit	• SupreDye BD3 Cycle Sequencing Kit • SupreDye dGTP BD3 Cycle	• BigDye Terminator v3.1 Cycle Sequencing Kit • BigDye dGTP Terminator v3.0 Cycle Sequencing Kit
Sequencing Reaction Cleanup	• ADS™ BD-XT Purification Kit, or • ADS™ Sequencing Reaction Cleaning Beads	Xterminator Purification Kit
Sequencing Polymer	PwrPOP™ P7	POP-7™
Sequencing Buffer	ADS™5x Sequencing Running Buffer	5x Sequencing Running Buffer

The standard protocol from Thermo Fisher Scientific was used for sequencing.

RESULTS AND DISCUSSION

SupreDye BD3 Chemistry Shows Better Dye Intensity and Uniformity Compared to Dye Chemistry from Vendor T Using the Regular Template

To compare the performance of the dye chemistries from AdvancedSeq and Vendor T, we first tested the two sequencing kits using the regular template with no secondary structure or high GC content. Sample preparation for sequencing – from sequencing reaction set-up, to cleanup, to capillary electrophoresis – was done according to the standard sequencing workflow from Thermo Fisher Scientific. Except for the SupreDye BD3 Cycle Sequencing Kits, all the other reagents were from Vendor T. The sequencing results shown in Figure 1 indicate that the SupreDye chemistry shows better signal intensity (stronger signals) and signal uniformity (slower signal decrease) compared to the dye chemistry from Vendor T. sWe then compared the performance of the two chemistries using the rest of sequencing reagents from AdvancedSeq and got similar results (data not shown).

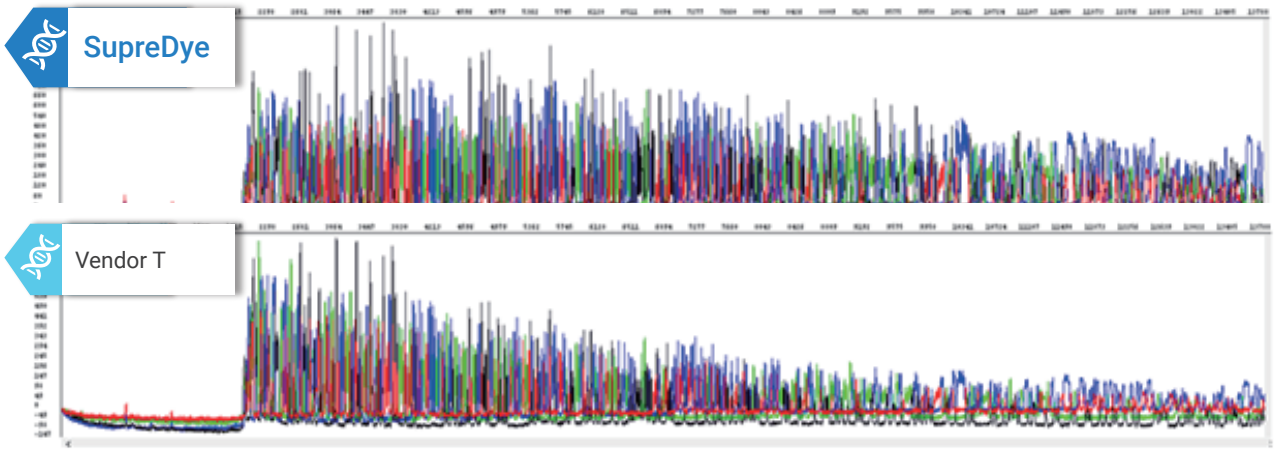

Figure 1. Sequencing performance comparison of SupreDye BD3 chemistry with dye chemistry from Vendor Ton a regular template.

SupreDye BD3 Chemistry Shows Better Performance than Dye Chemistry from Vendor T on a Difficult Template

Difficult templates with secondary structures are challenging for Sanger sequencing. Sequencing reaction becomes very critical for the success of sequencing these types of templates. SupreDye chemistries are optimized for difficult templates to generate high-quality sequencing results. As shown in Figure 2, SupreDye chemistry read through the secondary structure while the dye from vendor T dropped the signals at the secondary structure until the end of the sequencing read.

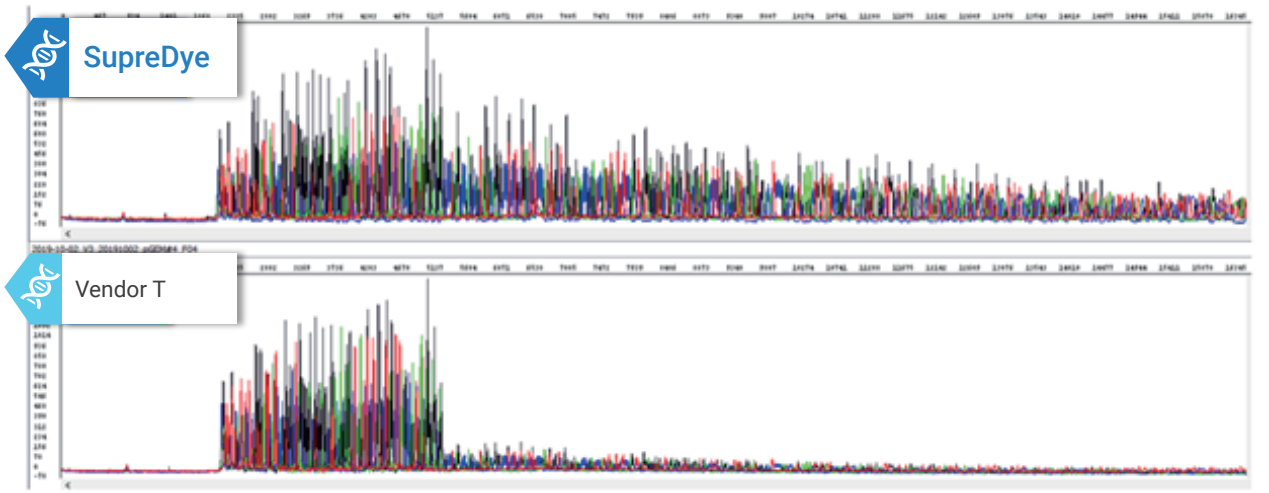

Figure 2. Sequencing performance comparison between SupreDye chemistry and dye chemistry from Vendor T on a difficult template.

Supredye dGTP BD3 Cycle Sequencing Kit Provides Better Sequencing Quality than SupreDye BD3 Cycle Sequencing Kit on High GC Templates

High GC templates are hard to be sequenced with sequencing kits designed for regular templates. The Supredye dGTP BD3 Cycle Sequencing Kit is optimized for high GC template sequencing. Figure 3 shows the comparison of performance between SupreDye dGTP BD3 and regular BD3 kits for sequencing high GC templates. It is obvious that the sequence read length and quality is much better with dGTP BD3 kit for the high GC template and should be used for sequencing high GC templates.

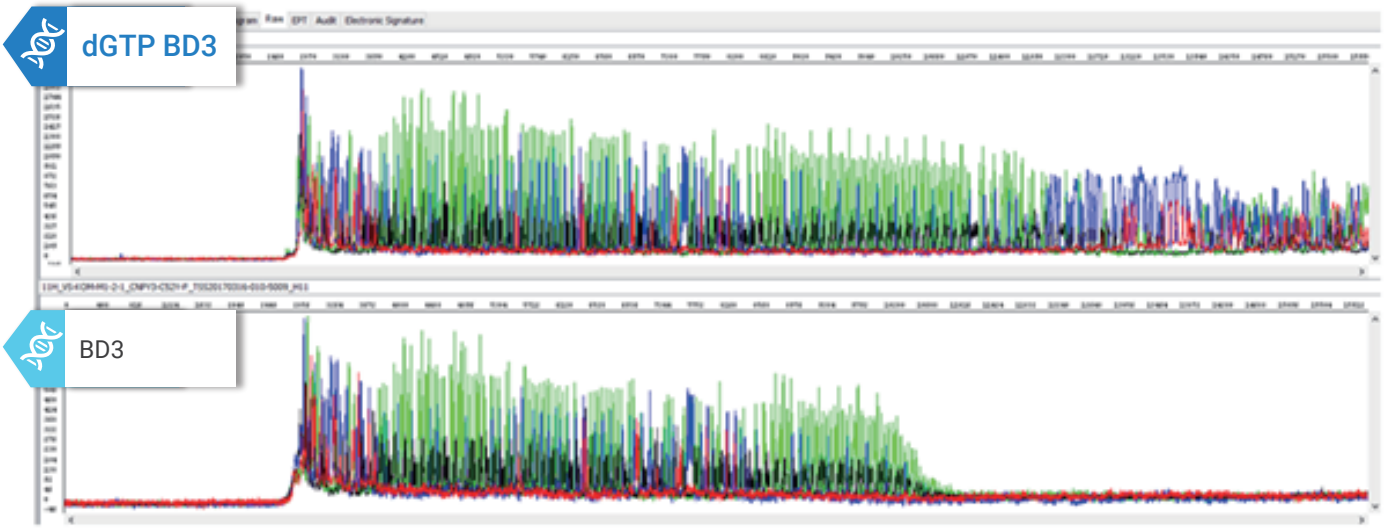

Figure 3. Sequencing performance comparison between the two AdvancedSeq sequencing kits on high -GC templates.

In conclusion, we have demonstrated the sequencing performance of the BigDye alternative, SupreDye BD3 Cycle Sequencing Kit, with regular and difficult templates by comparing with the sequencing kit from vendor T. For the high-GC template, the SupreDye dGTP BD3, as designed, shows better performance.