Faster Cycle Sequencing Applications Using the Applied Biosystems 9800 Fast Thermal Cycler

In a research environment that is sensitive to time and efficiency, reducing time-to-results is essential for discovery. The Applied Biosystems 9800 Fast Thermal Cycler significantly increases the speed at which PCR or cycle sequencing reactions can be performed. Several cycle sequencing applications have been tested on the 9800 Fast Thermal Cycler. Researchers using existing Applied Biosystems reagent sequencing kits can achieve sequencing read lengths and accuracy on the 9800 instrument comparable to those of the GeneAmp® PCR System 9700. Cycle sequencing times can also be reduced to only 50 minutes.

Figure 1. Reduce time-to-results.

Materials and Methods

Cycle sequencing testing on the 9800 Fast Thermal Cycler was performed with BigDye® Terminator v1.1 and v3.1 Cycle Sequencing Ready Reaction Kits, the dRhodamine Dye Terminator Cycle Sequencing Ready Reaction Kit, and the dGTP BigDye® Terminator v3.0 Cycle Sequencing Ready Reaction Kit. Several templates containing interesting, difficult-to-sequence motifs were used in the study. These motifs include GT repeats, G/C-rich, homopolymer T, homopolymer G, AGT-rich, secondary structure, TC repeats, and long GA stretches.

Note: *Although using GeneAmp® Fast PCR Master Mix on the 9800 Fast Thermal Cycler can reduce amplification time by half, the resulting PCR products may not be optimal for applications that require stringent sequencing quality.*

The reactions were formulated according to Tables 1–3 (next page) in MicroAmp® 96-Well Fast Thermal Cycling Plates, which were sealed with MicroAmp™ Clear Adhesive Film. Amplification and cycle sequencing were performed on the GeneAmp® PCR System 9700 and the Applied Biosystems 9800 Fast Thermal Cycler. The cycling protocols used are listed in Table 4 (9800 Fast modes) and Table 5 (9700 and 9800 Standard modes). Samples were purified by ethanol precipitation following the recommended protocols in the BigDye® Terminator v1.1 and v3.1 Cycle Sequencing Ready Reaction Kits. The 200-proof ethanol was obtained from Sigma-Aldrich (E702-3).

All samples (with the exception of dRhodamine samples) were resuspended in Hi-Di™ Formamide and sequenced on the Applied Biosytems 3730*xl* DNA Analyzer using the 50 cm Applied Biosystems Capillary Array and POP-7™ Performance Optimized Polymer. Samples sequenced using the dRhodamine Dye Terminator Cycle Sequencing Ready Reaction Kit were resuspended in Hi-Di™ Formamide and sequenced on the Applied Biosystems 3100 DNA Analyzer using the 50 cm Applied Biosystems Capillary Array and POP-6™ Performance Optimized Polymer.

TABLE 1

Sequencing reactions for MicroSeq® PCR products using BigDye® Terminator v3.1 and v1.1.

Reagents	Volume	Final Quantity
Primer (-21 M13)	1.0 µL	3.2 pmoles
Template	0.5 µL	10 ng
BigDye® Ready Reaction Mix	8.0 µL	
DiH_20	10.5 µL	
Total Reaction	20.0 µL	

TABLE 2

Sequencing reactions for BAC template with BigDye® v3.1.

Reagents	Volume	Final Quantity
Primer	1.0 µL	3.2 pmoles
Template	5.0 µL	1,000 ng
BigDye® Ready Reaction Mix	8.0 µL	
DiH_20	6.0 µL	
Total Reaction	20.0 µL	

TABLE 3

Sequencing reactions for difficult-to-sequence plasmids using BigDye® v3.1 and v1.1, dGTP BigDye® Terminator v3.0, and dRhodamine Dye Terminator.

Reagents	Volume	Final Quantity
Primer (-21 M13)	1.0 µL	3.2 pmoles
Template	1.0 µL	200 ng
Ready Reaction Mix	4.0 µL	
DiH_20	4.0 µL	
Total Reaction	10.0 µL	

TABLE 4

BigDye® Terminator v3.1 and v1.1, dGTP BigDye® v3.0 and dRhodamine

96°C	1 minute	
96°C	10 seconds	25X
50°C	5 seconds	
60°C	1 min, 15 sec	
4°C	Hold	
Run Time	50 minutes	

BAC DNA Specific BigDye® Terminator v3.1

96°C	2 minute	
96°C	20 seconds	50X
50°C	10 seconds	
60°C	2 minutes	
4°C	Hold	
Run Time	2 hours	

Fast mode protocols (cycle sequencing profiles for 9800 system in Fast mode).

TABLE 5

BigDye® Terminator v3.1 and v1.1

96°C	1 minute	
96°C	10 seconds	25X
50°C	5 seconds	
60°C	4 minutes	
4°C	Hold	
Run Time	2 hours	

dGTP BigDye® Terminator v3.0

96°C	10 seconds	25X
68°C	2 minutes	
4°C	Hold	
Run Time	1 hour	

dRhodamine Dye Terminator

96°C	10 seconds	25X
50°C	5 seconds	
60°C	4 minutes	
4°C	Hold	
Run Time	2 hours	

BAC DNA Specific BigDye® Terminator v3.1

96°C	5 minute	
96°C	30 seconds	50X
50°C	10 seconds	
60°C	4 minutes	
4°C	Hold	
Run Time	5 hours	

Standard mode protocols (cycle sequencing profiles for the 9700 and 9800 systems in Standard mode).

The MicroAmp™ Fast 96-Well Reaction Plate and other Fast accessories require special adapters for use with Applied Biosystems capillary electrophoresis instruments. Sequencing data was analyzed with Sequencing Analysis Software v5.2.

Note: *Applied Biosystems 3730 and 3730xl DNA Analyzers require Data Collection Software v3.0 or higher with Fast 96-Well Reaction Plates that have been heat-sealed. Septa-sealed plates can be used with either Data Collection Software v2.0 or v3.0. A full list of Applied Biosystems materials used in this study can be found in Table 8.*

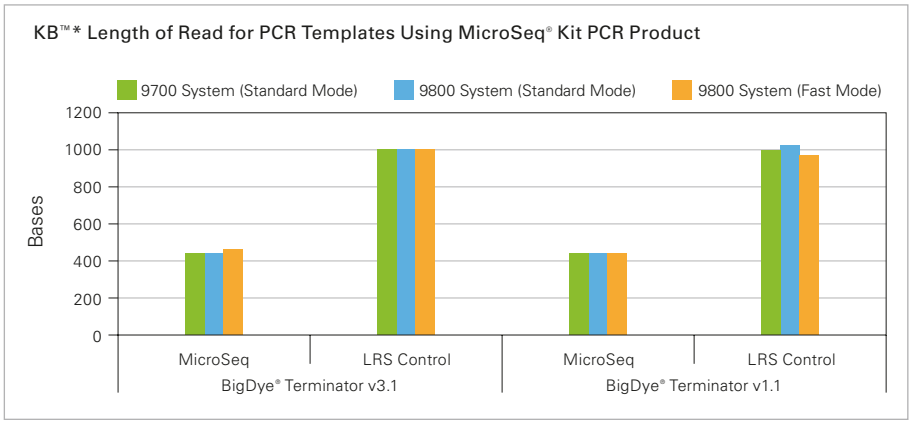

Figure 2. Comparable sequencing results on Fast and Standard mode. PCR product from the MicroSeq® Bacterial Identification Kit and LRS Control (BigDye® Terminator v3.1 Sequencing Standards) samples were used.

** KB™ Length of Read is defined as the usable high quality range of bases defined by the quality value metrics. The quality value of these samples was set at 20.*

Results

When used on the 9800 Fast Thermal Cycler, BigDye® Terminator v1.1 and v3.1 Cycle Sequencing Kits, the dGTP BigDye® Terminator v3.0 Cycle Sequencing Ready Reaction Kit, and the dRhodamine Dye Terminator Cycle Sequencing Ready Reaction Kit have been demonstrated to require less than half the time of the 9700 system. The KB™ Basecaller and Phred length-of-read (LOR) metrics (Figures 2, 4, 6, 8) indicate that the same Phred 20 LOR is achieved across multiple types of difficult-to-sequence motifs with the following instruments and cycling modes: the 9700 system in Standard mode, the 9800 system in Standard mode, and 9800 system in Fast mode.

Multiple experiments with several replicates confirmed the results for each sequencing template. The robustness of BigDye® Terminators v1.1 and v3.1 is retained on the 9800 Fast Thermal Cycler, which is demonstrated by the sample electropherograms (Figures 3, 5). The ability of dGTP chemistry kits to sequence through G-related difficult regions is uncompromised on the 9800 system.

Figure 7 shows an electropherogram of a long GT-repeat sequenced through with a dGTP BigDye® Terminator v3.0 Cycle Sequencing Ready Reaction Kit on the 9800 Fast Thermal Cycler. The dRhodamine Dye Terminator Cycle Sequencing Ready Reaction Kit, which is primarily used for sequencing cDNA or T-related difficult motifs, can also sequence through homopolymer T regions (Figure 9) without detectable enzyme slippage on the 9800 system.

Cycle Sequencing of PCR Products Using BigDye® Terminator v3.1 and v1.1 Kits

The data shown in Figure 2 were generated using three 96-well plates. The PCR product template was obtained using the Applied Biosystems MicroSeq® Bacterial Identification Kit, a PCR-based microbial identification system producing ~500-bp DNA fragments amplified from microbial 16s gene sequences. The long-

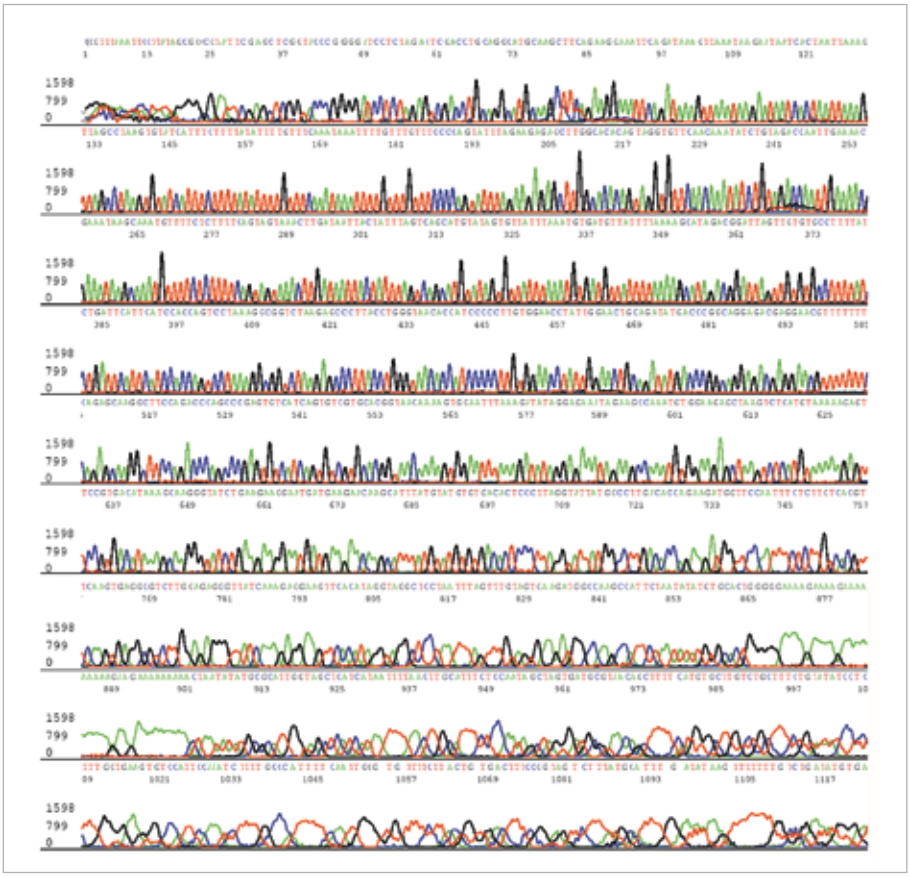

Figure 3. Sequencing of a BAC template on the 9800 Fast Thermal Cycler.

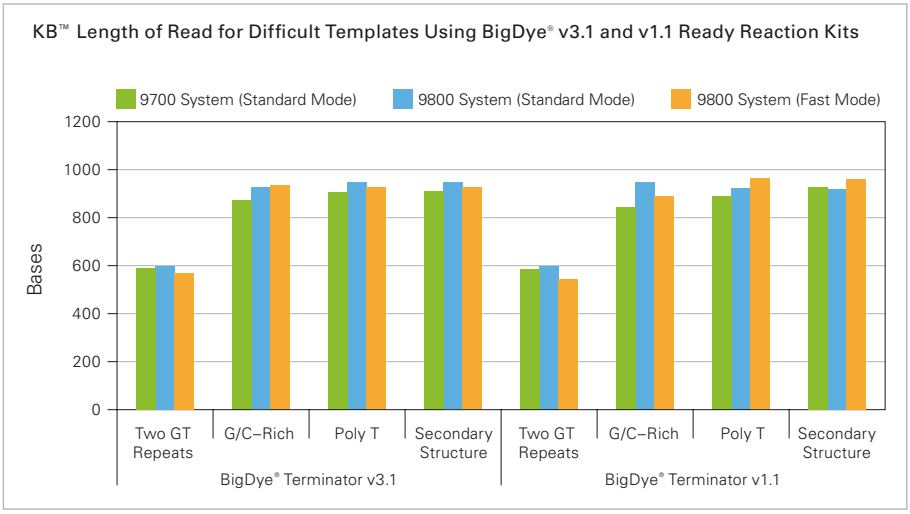

Figure 4. Comparable results achieved for Fast and Standards cycle sequencing when examining difficult-to-sequence plasmid templates.

read standard (LRS) controls (BigDye® Terminator v3.1 Sequencing Standards) were run as instrument control samples. These samples performed as expected on the 3730*xl* DNA Analyzer.

Cycle Sequencing of BAC DNA Using the BigDye® Terminator v3.1 Cycle Sequencing Kit

The data in Figure 3 were generated from a 96-well plate containing four replicates of the BAC template tested with the BigDye® Terminator v3.1 Cycle Sequencing Kit. The plate was cycle sequenced on the 9800 Fast Thermal Cycler in Fast mode. The LOR obtained was 1,093 bp. This is comparable to results obtained on the GeneAmp® PCR System 9700.

Cycle Sequencing of Difficult-to-Sequence Plasmid Templates Using BigDye® Terminator v3.1 and v1.1 Kits

The data in Figures 4 and 5 were generated from six 96-well plates, three of which were created with BigDye® Terminators v3.1 kits, and three with v1.1 kits. Each plate contained four replicates of each template tested. The read lengths were comparable across the three cycling modes and two instrument platforms.

Cycle Sequencing of Difficult-to-Sequence Template Using the dGTP BigDye® Terminator v3.0 Kit

The data in Figures 6 and 7 were generated from three 96-well plates, each of which contained four replicates of each template tested. The read lengths obtained from sequencing these difficult templates, using the three cycling modes and two instrument platforms, were comparable.

Cycle Sequencing of Difficult-to-Sequence Template Using dRhodamine Dye Terminator Kits

The data in Figures 8 and 9 were generated from three 96-well plates, each of which contained four replicates of each template tested. The read lengths obtained from sequencing these difficult templates, using the three cycling modes and two instrument platforms, were comparable.

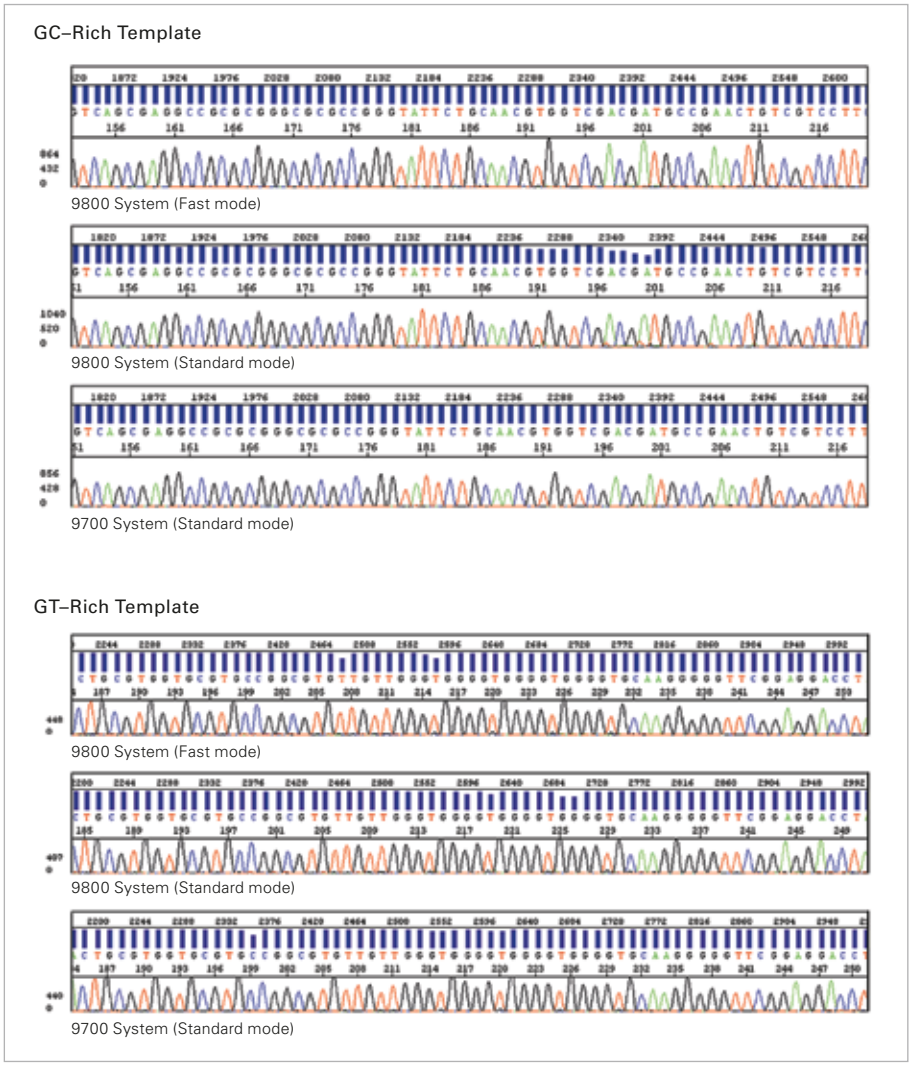

Figure 5. Electropherogram segments of difficult templates sequenced using BigDye® Terminator v3.1 Cycle Sequencing Kit.

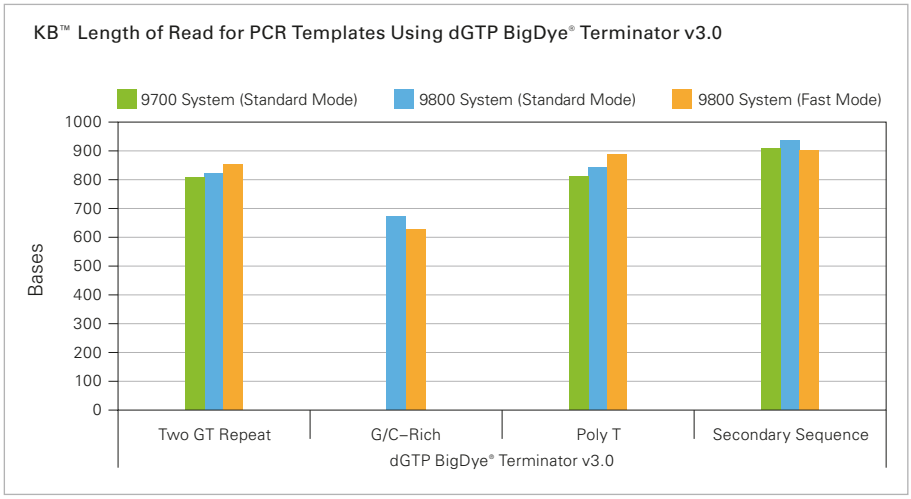

Figure 6. Comparable results achieved for Fast and Standard cycle sequencing when examining difficult-to-sequence plasmid templates using dGTP BigDye® Terminator v3.0.

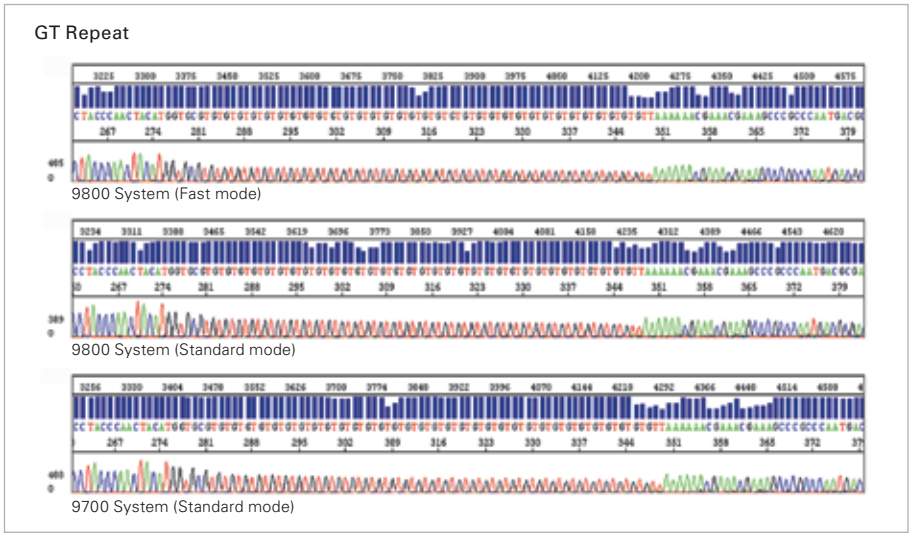

Figure 7. Electropherogram segments of a GT repeat sequence using dGTP BigDye® Terminator v3.0.

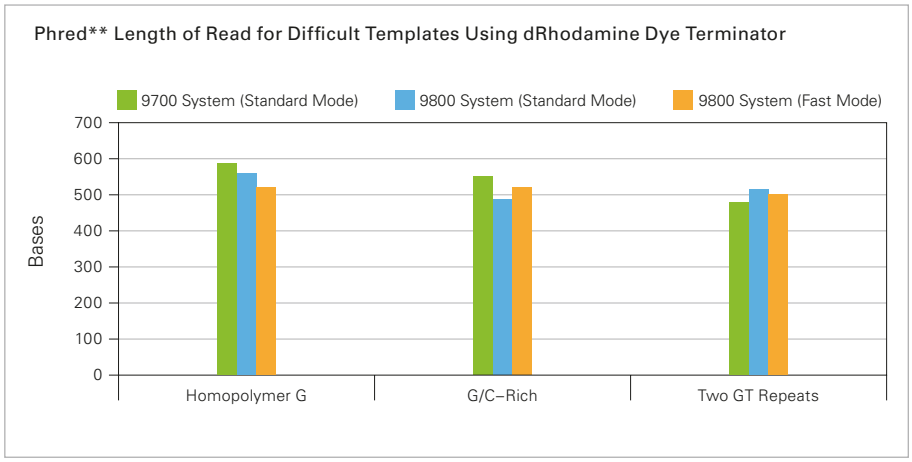

Figure 8. Comparable results achieved for Fast and Standard cycle sequencing when examining difficult-to-sequence plasmid templates using dRhodamine Dye Terminator.

** *Phred Length of Read = number of bases that a sliding 20–base window covers before the average Q score falls below Q=20 (base calling accuracy falls below 98.5%)*

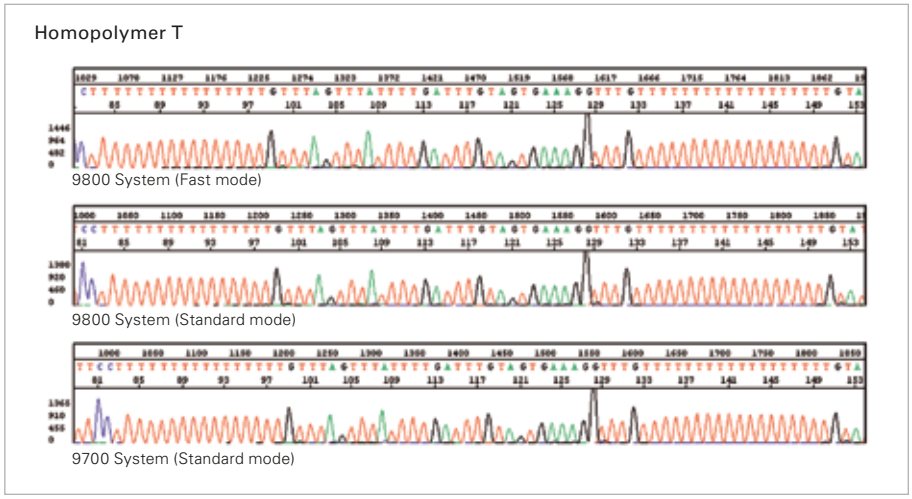

Figure 9. Electropherogram segments of a homopolymer T sequence using dRhodamine Dye Terminator.

Conclusion

In conclusion, faster cycle sequencing reactions can be generated with the Applied Biosystems 9800 Fast Thermal Cycler. The instrument delivers results from "real-world" templates that contain difficult-to-sequence regions such as GC-rich, homopolymer G, homopolymer T, and GT repeats. Results from the 9800 Fast Thermal Cycler are also comparable to standard-speed PCR and cycle sequencing results.

Because the 9800 Fast Thermal Cycler works seamlessly with BigDye® Terminator Kits, it is easily integrated into existing workflows and can significantly shorten the research process. Please contact Applied Biosystems Technical Support or your local sales representative for more information.

Note: *Use of the Fast 96-Well Reaction Plate on Applied Biosystems capillary electrophoresis instruments requires the use of special adapters. For 3730 and 3730xl instruments, Data Collection Software v3.0 is required when using Fast Reaction Plates that have been heat-sealed.*

Septa-sealed plates may be used with either Data Collection Software v2.0 or v3.0. The use of reagents at concentrations other than those recommended by Applied Biosystems may yield different results. PCR products in the above-referenced experiments were generated at standard speeds.

ORDERING INFORMATION

Product Description	Quantity	Part Number
Thermal Cycling		
GeneAmp® PCR System 9700		4314879
Applied Biosystems 9800 Fast Thermal Cycler		4352604
GeneAmp® Fast PCR Master Mix (2X) with Protocol	250 Reactions	4362070
AmpliTaq Gold® PCR Master Mix	250 Units	4318739
BigDye® Terminator v1.1 Cycle Sequencing Kit	100 Reactions	4337450
BigDye® Terminator v3.1 Cycle Sequencing Kit	100 Reactions	4337455
dRhodamine Dye Terminator Cycle Sequencing Ready Reaction Kit	100 Reactions	403044
dGTP BigDye® Terminator v1.0 Cycle Sequencing Ready Reaction Kit	100 Reactions	4307175
Sequencing		
Applied Biosystems 3730*xl* DNA Analyzer		3730*xl*
POP-6™ Polymer for 3100/3100–*Avant*™ Genetic Analyzers	7 mL	4316357
POP-7™ Polymer for 3730/3730*xl* DNA Analyzers	1 x 28 mL	4363929
Applied Biosystems 3730*xl* DNA Analyzer Capillary Array, 50 cm	1 Array	4331246
Plastics and Accessories		
MicroAmp™ Fast 96-Well Reaction Plate	10 Plates	4346907
MicroAmp™ Clear Adhesive Film	100 Films	4306311
ABI PRISM® 310 Genetic Analyzer Fast Plate Adapter†	1 Adapter	4370141
ABI PRISM® 3100/Applied Biosystems 3130 Genetic Analyzers		
Autosampler Plate Adapter Kit*	1 Kit	4367468
96-Well Fast Plate (0.1 mL) Base	4-Pack	4367470
96-Well Fast (0.1 mL) Plate Retainer	4-Pack	4367471
Applied Biosystems 3730/3730*xl* DNA Analyzers		
96-Well Fast (0.1 mL) Plate Base (Septa)†	4-Pack	4367469
96-Well Fast (0.1 mL) Plate Retainer (Septa)†	4-Pack	4367472
96-Well Fast (0.1 mL) Plate Base (Film)†	4-Pack	4367473
96-Well Fast (0.1 mL) Plate Retainer (Film)†	4-Pack	4367474

* Includes one plate base and one plate retainer, a box of plates, and a box of sealing Septa.

† Available late 2006

Printed in the USA. 09/2010 Publication 104AP01-02

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