

New Advances in DNA Sequencing Chemistry

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BigDye® Terminator v3.1 and v1.1 Cycle Sequencing Kits

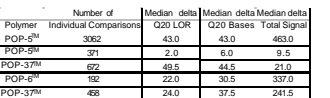
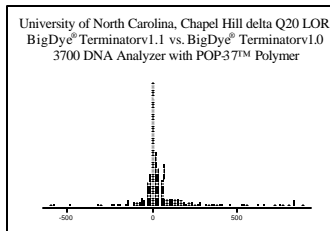
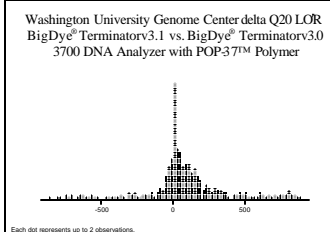
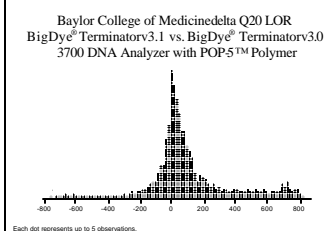
Fluorescent dideoxy sequencing has advanced greatly since the early days of automated DNA sequencing about 15 years ago. Cycle sequencing using thermally stable DNA polymerase enzymes provided a robust method of sequencing templates over a much wider and lower range of concentrations than was previously possible. Fluorescent dideoxy terminators reduced the number of steps, equipment and supplies required to perform sequencing reactions, and BigDye® terminators provided several fold additional sensitivity. These advances greatly facilitated the rapid sequencing of the human genome. Researchers today are challenged to finish the difficult-to-sequence areas of the human genome and other organisms, and to obtain highly accurate sequences of individuals in studying polymorphisms. We are now introducing a new generation of sequencing reagents to continue aiding the research community. In this presentation we describe these improvements, and compare sequencing results from a range of different templates and conditions with existing products.

BigDye® Terminator v1.1 and v3.1 Cycle Sequencing Kits Characteristics

- BigDye® Terminator v1.1 and v3.1 are reformulations of BigDye® Terminator v1 and v3 chemistries
- No new software or instrument recalibration required for use*
- Optimized protocols will enhance performance
- Sequencing buffer formulated specifically for BigDye® Terminator v1.1 and v3.1 Cycle Sequencing kits.

*Note: Assumes instrument currently has corresponding BigDye® chemistry calibration files

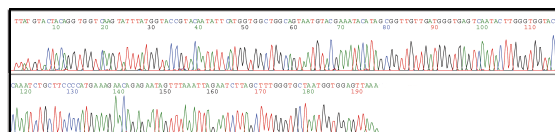
Test sites were asked to sequence the same templates with BigDye® Terminator v3.1 kit and BigDye® Terminator v3.0 kit (or v1.1 kit and v1.0 kit). The data on this page are the results from four of those comparisons.



Test Site	Comparison	Instrument	Polymer	Number of Individual Comparisons	Median Q20 LOR	Median delta Q20 LOR	Median delta Total Signal
Baylor College of Medicine	v3.1 kit vs. v3.0 kit	3700 DNA Analyzer	POP-6™ Polymer	3002	43.0	43.0	463.0
University of Utah	v3.1 kit vs. v3.0 kit	3700 DNA Analyzer	POP-6™ Polymer	371	2.0	6.0	9.5
Washington University	v3.1 kit vs. v3.0 kit	3700 DNA Analyzer	POP-6™ Polymer	622	49.5	44.5	21.0
University of North Carolina Chapel Hill	v1.1 kit vs. v1.0 kit	3100 DNA Analyzer	POP-6™ Polymer	50	22.0	30.5	337.0
University of North Carolina Chapel Hill	v1.1 kit vs. v1.0 kit	3700 DNA Analyzer	POP-3™ Polymer	48	24.0	37.5	241.5

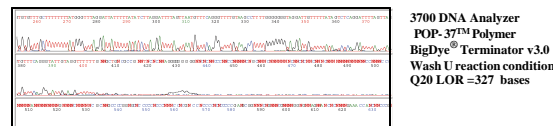
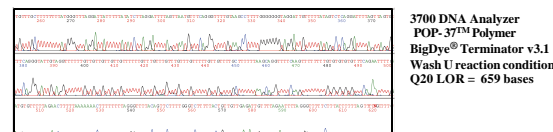
Confirmation Test Site Data

Short PCR product sequencing with BigDye® Terminator v1.1.



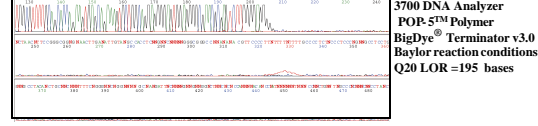
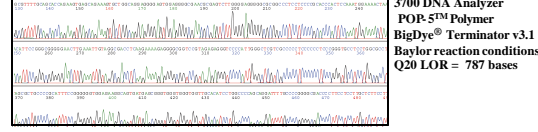
The v1.1 kit successfully sequences a short PCR product generated from human mitochondrial DNA. The PCR product shows 100% basecalling accuracy beginning with the first base adjacent to the primer. This sample was run on the 3100 Genetic Analyzer using POP-6™ Polymer.

BigDye® Terminator v3.1 compared to BigDye® Terminator v3.0 on ABI PRISM® 3700 DNA Analyzer with POP-37™ Polymer sequencing a poly T repeat element



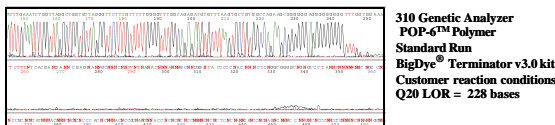
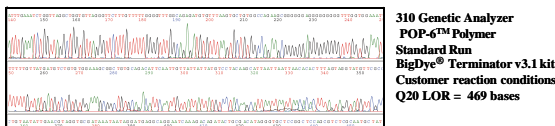
Data Courtesy of Dr. Elaine Mardis, Washington University Genome Center

BigDye® Terminator v3.1 compared to BigDye® Terminator v3.0 on ABI PRISM® 3700 DNA Analyzer with POP-5™ Polymer sequencing a G/A specific stop

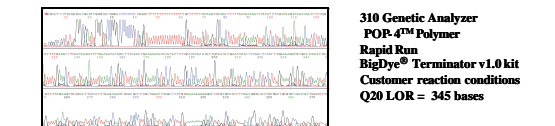
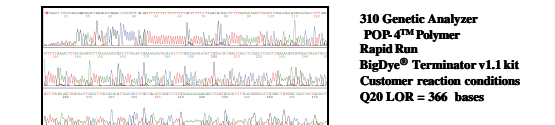


Data Courtesy of Donna Muzny, M.Sc and Graham Scott, Ph.D, Human Genome Sequencing Center, Baylor College of Medicine

BigDye® Terminator v3.1 compared to BigDye® Terminator v3.0 on ABI PRISM® 310 Genetic Analyzer with POP-6™ Polymer Standard Run

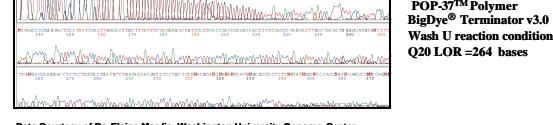
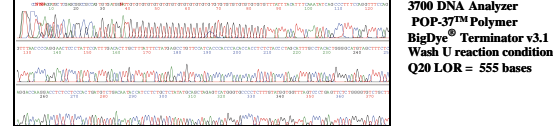


BigDye® Terminator v1.1 compared to BigDye® Terminator v1.0 on ABI PRISM® 310 Genetic Analyzer with POP-4™ Polymer Rapid Run

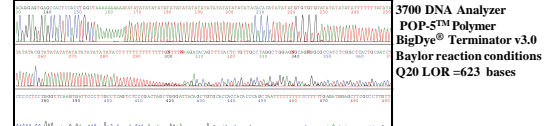
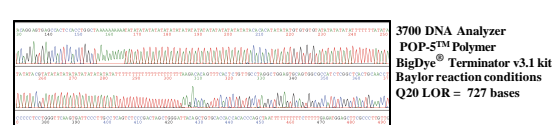


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BigDye® Terminator v3.1 compared to BigDye® Terminator v3.0 on ABI PRISM® 3700 DNA Analyzer with POP-37™ Polymer sequencing a G/T repeat element



BigDye® Terminator v3.1 compared to BigDye® Terminator v3.0 on ABI PRISM® 3700 DNA Analyzer with POP-5™ Polymer sequencing a dinucleotide repeat and homopolymer regions



Data Courtesy of Donna Muzny, M.Sc and Graham Scott, Ph.D, Human Genome Sequencing Center, Baylor College of Medicine

Removal of Unincorporated Dye Terminators

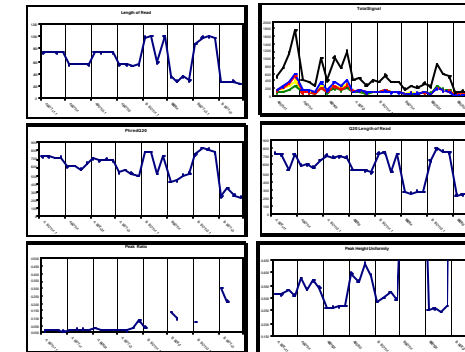
If you are using:	Formulation	Clean up	We recommend that you use:	Formulation	Clean up
v1.0 kit	Ethanol		v1.1 kit	Ethanol/EDTA	
v2.0 kit	Ethanol		v1.1 kit	Ethanol/EDTA	
v3.0 kit	Ethanol		v3.1 kit	Ethanol/EDTA	
v1.0 kit	Isopropanol		v1.1 kit	Ethanol/EDTA	
v2.0 kit	Isopropanol		v1.1 kit	Ethanol/EDTA	
v3.0 kit	Isopropanol		v3.1 kit	Ethanol/EDTA	
v1.0 kit	Ethanol/Sodium Acetate		v1.1 kit	Ethanol/EDTA/Sodium Acetate	
v2.0 kit	Ethanol/Sodium Acetate		v1.1 kit	Ethanol/EDTA/Sodium Acetate	
v3.0 kit	Ethanol/Sodium Acetate		v3.1 kit	Ethanol/EDTA/Sodium Acetate	
v1.0 kit	Plate and Spin Column without SDS		v1.1 kit	Plate and Spin Column without SDS	
v2.0 kit	Plate and Spin Column without SDS		v1.1 kit	Plate and Spin Column without SDS	
v3.0 kit	Plate and Spin Column with SDS		v3.1 kit	Plate and Spin Column with SDS*	

* SDS necessary if you are using 8 -> 20 ul Ready Reaction mix per reaction.

Removal of Unincorporated Terminators Using Ethanol/EDTA Precipitation

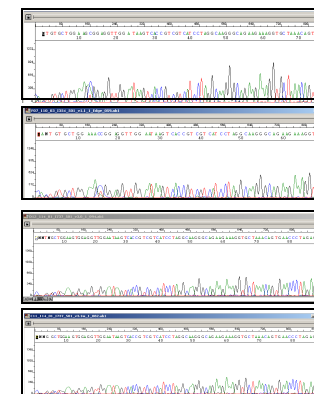


BigDye® Terminator v1.0 8 ul Ready Reaction mix in 20 ul reaction
BigDye® Terminator v1.1 8 ul Ready Reaction mix in 20 ul reaction
BigDye® Terminator v3.0 8 ul Ready Reaction mix in 20 ul reaction
BigDye® Terminator v3.1 8 ul Ready Reaction mix in 20 ul reaction



Template A= G/C rich template Template B= G/A, G/T motif

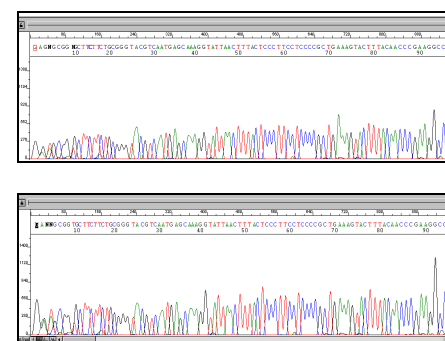
Removal of Unincorporated Terminators Using Performa® DTR 96 well Columns*



BigDye® Terminator v1.0 8 ul Ready Reaction mix in 20 ul reaction
BigDye® Terminator v1.1 8 ul Ready Reaction mix in 20 ul reaction
BigDye® Terminator v3.0 8 ul Ready Reaction mix (cleanup with SDS)
BigDye® Terminator v3.1 8 ul Ready Reaction mix in 20 ul reaction (cleanup with SDS)

*Performa® DTR 96 well column from Edge Biosystems

Removal of Unincorporated Terminators from BigDye® Terminator v3.0 and BigDye® Terminator v3.1 Reactions Using CleanSeq™ kit*



BigDye® Terminator v3.0 8 ul Ready Reaction mix in 20 ul reaction
BigDye® Terminator v3.1 8 ul Ready Reaction mix in 20 ul reaction

*CleanSeq™ kit product of Agencourt

Analytical Tools Used in the Development of BigDye® Terminator v1.1 and v3.1

- Length of Read (LOR) - Number of continuous bases that are called correctly (based on a known sequence) until a total error of 1.5% is reached.
- Q20 Length of Read (Q20 LOR) - Number of continuous bases in which the average Phred quality score does not drop below 20 in a sliding window of 21 bases.
- Phred Q20 - Total number of bases with a Phred quality score of 20 or above.
- Peak Ratio - Average ratio of the signals of the base-called peaks to the signals of any uncalled peaks underneath them.
- Peak Height Uniformity - Deviation of the peak heights from the average local peak height on a per color basis.
- Total Signal - Total average signal from all 4 bases.

Conclusions

BigDye® Terminator v1.1 and v3.1 Cycle Sequencing kits offer improved robustness, optimized signal balance and greater peak height uniformity. We have illustrated how these improvements result in longer lengths of read and higher success rates. These formulations should allow researchers to complete experiments in less time, with fewer runs, thus lowering the cost of sequencing projects

Future

In a continuing effort to facilitate accurate and consistent sequencing for the research community we have ongoing efforts to simplify work flow and improve robustness, stability, and utility of our reagents. We are considering reformulations of other Applied Biosystems Cycle Sequencing kits such as the BigDye® Terminator dGTP kit using the benefits derived from BigDye® Terminator v1.1 and v3.1 Cycle Sequencing kits research.

Acknowledgement

Everyone in the research group is very grateful for the support of Mark Garret in our Service group for keeping our instruments in top condition. We would also like to thank Bob Ruhfel, Mike Sklar and Paolo Vatta for help generating and presenting data.