

A New Purification Method for DNA Sequencing Reactions

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ABSTRACT

BigDye® XTerminator™ Kit is a new single-tube method for purifying DNA sequencing reactions prior to electrophoretic analysis. Sequencing reactions purified using this method display very few artifacts from residual dye-labeled nucleotides (dye blobs) as well as excellent recovery of the smallest extension products. BigDye XTerminator also has unique workflow advantages over conventional purification schemes such as ethanol precipitation or spin column purifications. Performance of BigDye XTerminator with a variety of DNA sequencing reactions will be demonstrated. Workflow and automation issues will also be described.

INTRODUCTION

DNA sequencing reactions are enzymatic reactions which generate a continuum of fluorescently-labeled DNA fragments from 15nt to over 1200nt. DNA sequencing reactions contain buffer salts, metal ions, polymerase, nucleotides, oligonucleotide primers, and sometimes other chemical additives. Finished sequencing reactions contain residual dye-labeled ddNTPs (terminators) that must be removed prior to electrophoretic analysis on a DNA sequencer. Dye-labeled terminators are charged and can electrophorese through the capillary with the DNA sequencing fragments. Failure to completely remove dye-labeled terminators results in dye blobs that cause errors in DNA sequencing (Figure 3). A second requirement of DNA sequencing purification is that the sample be desalted. The second requirement is imposed because capillary sequencers use electrokinetic injection as a means to introduce samples. Salt concentration in excess of 100uM is known to inhibit injection of DNA sequencing fragments on capillary sequencers.

The BigDye® XTerminator™ Purification Kit sequesters sequencing reaction impurities including salt ions, unincorporated dye terminators, and dNTPs to prevent their co-injection with dye labeled extension products. BigDye XTerminator reagents are added directly to finished sequencing reactions and mixed. During mixing, the BigDye XTerminator reagents capture and immobilize unwanted components. After mixing, the reactions are briefly centrifuged to move the insoluble fraction of the reagent mixture and the captured reaction components to bottom of the reaction well. The purified dye-labeled extension products remain in the supernatant and are injected directly from the supernatant into the DNA sequencer using specialized run modules.

MATERIALS AND METHODS

BigDye® XTerminator™ Purification Kit, MicroAmp™ Optical 96 and 384-Well Reaction Plates, 3130xl Genetic Analyzer, 3730 DNA Analyzer, BigDye® Terminator v3.1 Cycle Sequencing Kit, Sequencing Analysis software v.5.2. (Applied Biosystems, Foster City, CA, USA),

Centri-Sep columns (Princeton Separations, Adelphia, NJ, USA),

ALPS-300 Plate Sealer (ABgene, Rochester, NY, USA)

Centrifuge Model 5403 (Eppendorf, Westbury, NY, USA)

Digital Vortex-Genie 2 (Scientific Industries, Bohemia, NY, USA)

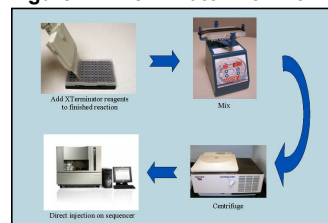
Biomek FX workstation (Beckman Coulter, Fullerton, CA, USA)

Bubble Paddle Reservoir (V&P Scientific, San Diego, CA, USA)

Vertical Shaker (Union Scientific, Randallstown, MD, USA)

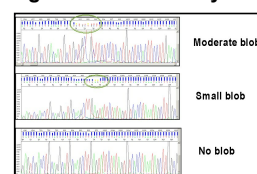
RESULTS

Figure 1. XTerminator workflow



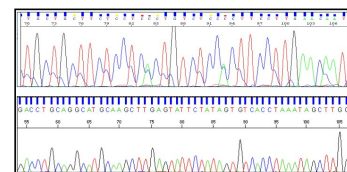
BigDye XTerminator utilizes a simple workflow. Reagents are added to the same well used for thermal cycling and are mixed. The XTerminator reagent binds impurities and leaves purified DNA in solution. The DNA can then be directly injected (figure 2).

Figure 3. Residual dye blobs



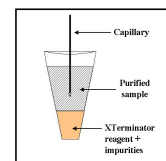
Blobs range in severity from severe to minimal. Severe blobs can result in miscalled bases. Small blobs often do not effect the basecalling accuracy, but can lower the quality of basecalling. BigDye XTerminator-purified samples resulted in data with no blobs for the majority of DNA sequencing templates that were tested.

Figure 5 - Enhanced stability



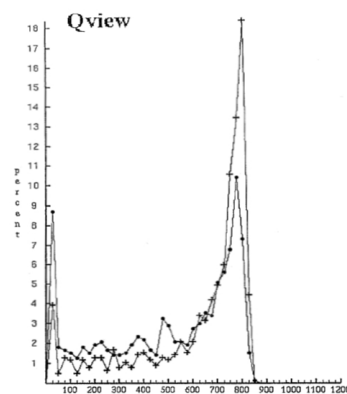
Samples purified with BigDye XTerminator are stable at room temperature. The top trace shows a sample stored 24 hours in DI water displaying signs of sample breakdown. The bottom trace shows a sample purified with XTerminator and stored for 48 hours. There was no sign of breakdown with the XTerminator-purified sample.

Figure 2. Direct injection using BigDye XTerminator



Direct injection of purified sample from the same well used for thermal cycling and purification is enabled with BigDye XTerminator. XTerminator reagents remain in the sample tube during electrokinetic injection.

Figure 4. Read Length vs. ethanol precipitation*



Series	Sym	Reads	McBin	MxBin	%100	%400	Avg	Q20
BigDye XTerminator	+	768	800	825	6.9	81.64	637	4.6e+5
Ethanol	•	768	775	850	13.8	67.06	553	3.7e+5

Legend Key

McBin: The most common bin

MxBin: Bin of reads with the longest read lengths

%100: Percent of reads with 100 or fewer Q-20 bases

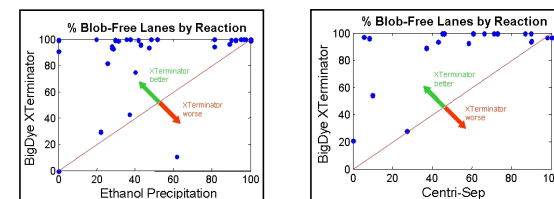
%400: Percent of reads with 400 or fewer Q-20 bases

Avg: Average % of Q-20 bases (where #Q-20 bases >=100)

Q20: Total # of Q-20 bases (where #Q-20 bases >=100)

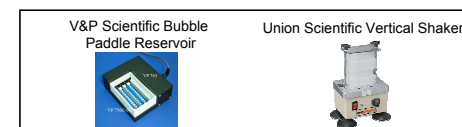
* Evaluation data and graphic courtesy of Washington University Genome Sequencing Center, St. Louis, MO, USA

Figure 6. Blob removal: Comparison with ethanol precipitation and Centri-Sep columns



A variety of sample templates were tested using BigDye XTerminator including plasmids and PCR products of various sizes and input concentrations. Results demonstrate that BigDye XTerminator is comparable or superior to Centri-Sep and ethanol precipitation purifications in terms of dye blob removal.

Figure 7. BigDye XTerminator in high throughput labs



BigDye XTerminator fits easily into high throughput environments. Using the Beckman FX platform equipped with a V&P Scientific stirrer we were able to achieve rapid plate filling (Figure 9 below). Following dispensing, (18) 384-well plates or (6) 96-well plates were simultaneously mixed using the Union Scientific Vertical Shaker.

Figure 8. Automated plate filling throughput

	Minutes per 18 plates		Plates per hour	
	96-well plates	384-well plates	96-well plates	384-well plates
96-well head	5.2 min	9.0 min	209 plates	120 plates
384-well head	n/a	5.2 min	n/a	209 plates

Measured using Beckman FX equipped with V&P Scientific Bubble Paddle Reservoir.

CONCLUSIONS

BigDye® XTerminator™ has been shown to be an effective method for purification of DNA sequencing reactions prior to electrophoretic analysis. The performance as measured by dye blob removal & read length was shown to be comparable or superior to conventional purification methods. Samples purified using BigDye XTerminator are stable at room temperature for 48 hours at room temperature and up to 10 days at 4°C. BigDye XTerminator has considerable workflow advantages over conventional purification techniques as is easily adapted for use in high throughput automated workflows.

TRADEMARKS/LICENSING

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