

A Novel Method for Sequencing Reaction Purification

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ABSTRACT

The presence of unincorporated dye terminator and poly-ionic salts present in unpurified reaction mixtures interferes extensively with the CE separation of sequencing reaction extension products. Physical removal of these reaction components, using size exclusion chromatography or ethanol precipitation, has traditionally been performed in order to satisfy goals for signal yield, uniformity and quality. We present here an alternative approach that involves effectively sequestering unwanted reaction components to exclude their co-injection with extension products. The purification is performed in the same well as the sequencing reaction, thus reducing sample handling steps. Adaptation of this purification method can therefore result in significantly enhanced workflow. When compared to other approaches to sequencing reaction purification, this method yields comparable or superior sequencing signal quality. An additional benefit derived from adaptation of this approach is enhanced sample stability. We further show that this novel method is flexible and can be effectively integrated into the workflow of most sequencing laboratories.

INTRODUCTION

Nucleic acid sequencing has come to be viewed as an extended process involving a multi-component workflow that begins with sample preparation and ends with generation of annotated sequence data. Each element of the process involved in sequencing has become subject to increased scrutiny in order to satisfy increased demands for efficiency and throughput.

Post sequencing reaction purification represents a significant feature of the overall workflow. Reaction cleanup has been characterized by time consuming methods involving multiple fluid transfer steps and technique-sensitive methods that often yield results that lack uniformity.

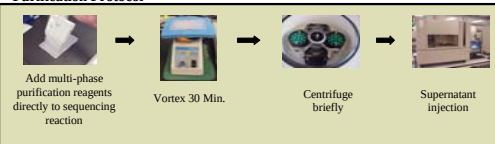
Applied Biosystems has recently developed a kit for preparing sequencing reactions for capillary electrophoresis. The BigDye Xterminator kit has been designed to provide superior performance and employ a simplified, time saving protocol. The kit's design criteria specifies that performance, as measured by: 1) Dye artifact removal, 2) Retention of short extension products, 3) Overall signal strength, and 4) Length of read, be equal to or greater than that observed for existing methods.

Coupled with the elimination of fluid transfer steps, simplified workflow and a significant reduction in technique sensitivity, the BigDye Xterminator kit achieves its design goal of streamlining the sequencing process workflow.

MATERIALS AND METHODS

- Purification multi-phase reagent added to sequencing reactions
- Impurities bound to particle phase
- Extension products remain in solution
- Multi-phase reagent is *not removed* from sample
- Direct injection from supernatant
- Purification product consists of:
 - Multi-phase reagent
 - Aqueous additive mixture

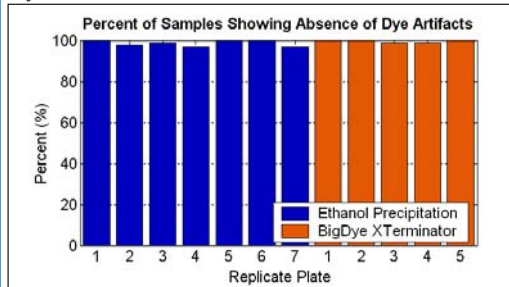
Purification Protocol



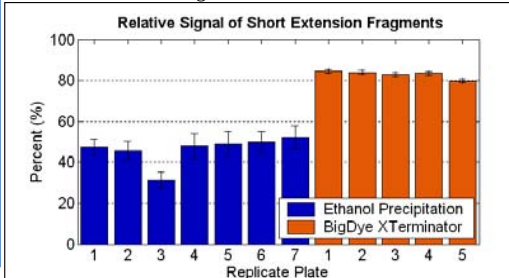
- Vortex 20-30 min, minimal hands-on time.
- Short total purification process time.
- Immediate load onto CE platform.
- Amenable to laboratory automation
- Alternative protocol available to permit batch processing

RESULTS

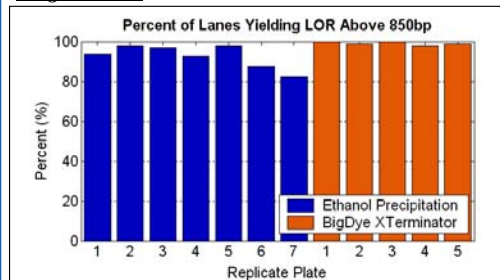
Dye Artifact Removal



Retention of Small Fragments

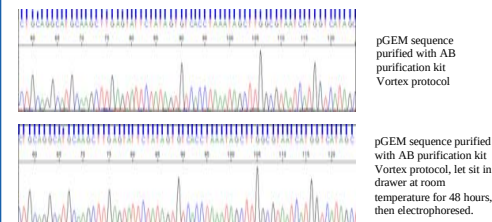


Length of Read



Reaction Stability

In addition to ensuring good quality lengths of read and complete removal of unincorporated dye terminators, we have also tested for sample stability. Below are data from sequencing reactions left at room temperature for 48 hours and protected from light prior to electrophoresis.



Sequencing reactions left at room temperature for 48 hours did not show signs of dye degradation.

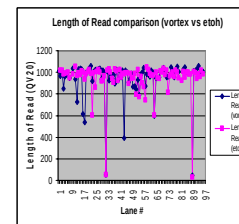
EXTERNAL TEST SITE RESULTS

Select external sites have participated in the evaluation of the BigDye Xterminator Purification Kit. Kit performance was evaluated using samples typical of their production sequencing operation and the results compared to those obtained using their current sequencing reaction purification protocol. Purified reactions were then separated using an Applied Biosystems 3730xl DNA Analyzer with modified run modules. Below are representative analysis of these test site data with the following metrics:

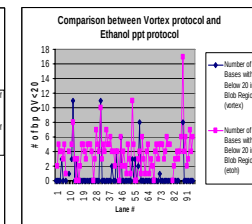
Length of Read (QV 20): Generated by the KB™ Basecaller software.

'Blob Number': The number of bases in the dye blob region with QV < 20. A value of 'zero' means there are no detectable dye blobs using the Q20 metric.

Q20 Length of Read



Dye Artifact Removal



Data courtesy of Baylor College of Medicine Human Genome Sequencing Center. Data compares the Q20 length of read and 'blob number' on replicate production samples that are purified by either AB's Purification Kit or current ethanol precipitation method. Results show comparable length of read between the two methods. However, AB's Purification Kit samples show significant improvement in dye blob removal.

CONCLUSIONS

Applied Biosystems' BigDye Xterminator kit is a novel post sequencing reaction purification kit that offers a rapid and simple solution to post sequencing reaction purification. Performance data shown demonstrates that performance compares favorably with ethanol precipitation reflecting through dye artifact removal, superior retention of small fragments, enhanced signal strength and uniformly long read lengths. Use of BigDye Xterminator streamlines the sequencing process and integrates easily into existing workflows.

ACKNOWLEDGEMENTS

- The entire research team is very grateful for the help and support by David Rodriguez in providing us with expert knowledge in automation and robotics systems.
- We would also like to thank our test sites for evaluating this kit and providing valuable feedback to guide the further development of this purification kit.

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