

High throughput sequence reaction cleanup using Millipore Montage SEQ₉₆ Sequencing Reaction Cleanup Kit* and Beckman Coulter's Biomek® FX Laboratory Automation Workstation equipped with ORCA® robotic arm

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ABSTRACT

The information included in this paper describes the utilization of the liquid handler Biomek FX Laboratory Automated Workstation, equipped with ORCA® robotic arm, in the in the removal of the sequencing dye using Millipore Montage SEQ₉₆ Sequencing Reaction Cleanup Kit. Using this system, sequence reactions are purified, from salts and unincorporated dye terminators, by vacuum-based size exclusion separation technology. Finally the DNA was recovered, by pipetting resuspension, from the membrane and transferred in a ready to use sequencing plate. A specific protocol was designed and validated in SAMI® software to use the kit on the automated core system. The following system components for the sequence reaction cleanup will be described:

- The automated Core System workstation: Biomek FX & ORCA
- The software and method to drive the workstation
- The results when purifying sequences reactions using Montage SEQ₉₆ Kit

Representative data obtained from the purification of sequences reactions using this system will be shown. Data from capillary sequencing on Applied Biosystems 3730 DNA Analyzer will be shown demonstrating the suitability of the cleaned reactions for capillary sequencing.

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INTRODUCTION

Methodology for removal of the sequencing dye using Millipore Montage SEQ₉₆ Sequencing Reaction Cleanup Kit was developed for the Biomek FX, an automated liquid handler from Beckman Coulter Inc. equipped with ORCA Robotic arm and Plates Carousel, (see Figure 1). This platform provides sufficient deck space to process one plate at a time, until to a maximum of sixteen plates, without user interaction after initial setup. Furthermore, the Biomek Software allows for user-defined variables to be included in the method. Using our settings, a 96-well plate can be processed in approximately 14 minutes using only the liquid handler; using the complete core system (BFX, ORCA and Carousel) two 96-well plates can be processed in approximately 34 minutes, four in 69 minutes up to 16 plates in 280 minutes.

The results presented here demonstrate the quantity and quality of purified sequence reactions obtained using Millipore Montage SEQ₉₆ Sequencing Reaction Cleanup Kit reagents on the Biomek FX. Cleaned reactions was tested on the Applied Biosystems 3730 DNA Analyzer.

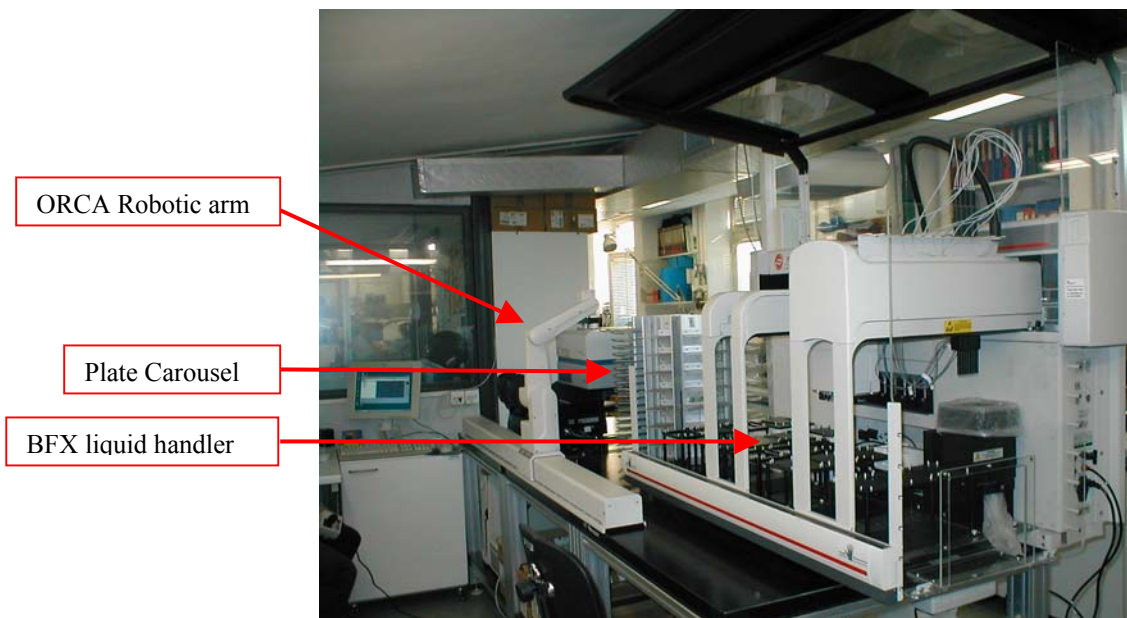


Figure 1 The automated Core System workstation: Biomek FX, plates carousel & ORCA

MATERIALS AND METHODS

Sequence reactions were performed with a 1/20x BigDye Terminator 3.1 dilution in comparison to the recommended protocol using a final volume reaction of 20 μ L. The same reactions plate was split in two and the dye removal was performed, on the first plate, manually as recommended by Applied Biosystems, and on the other plate in automation using the Biomek FX dual bridge (see Figure 1) with Millipore kit. As template were used plasmids (pBluescript (+)) and PCR products. Hardware needed to purify reactions on this platform includes a Gripper tool, a vacuum manifold and a blotter. Used labware is removed from the worksurface of the instrument with ORCA. The filtration plate is used to purify, from salts and unincorporated dye terminators, the sequence reactions using a vacuum-based size exclusion separation technology. Finally the DNA was recovered, by pipetting resuspension, from the membrane and transferred in a ready to use sequencing plate.

The Biomek FX protocol is edited and controlled via Biomek Software, a common software architecture among Beckman Coulter's Laboratory Automation Workstations. Software features include variables, version control, and sample tracking. The whole integrated core system (BFX, ORCA and Carousel) is controlled via SAMI Software (made by Beckman Coulter) using which we was able to build method to process up to 16 unattended plates. A view of the Biomek method (see Fig. 2) and SAMI method (see Fig. 3) are shown below:

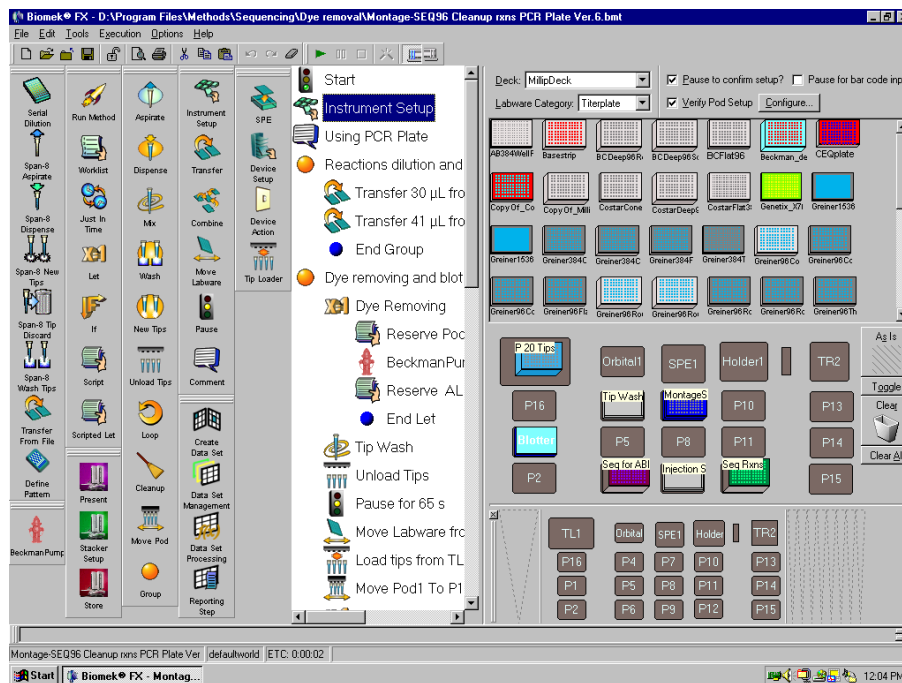


Figure 2 The dye removal method on the Biomek FX



Figure 3 The dye removal method on SAMI software

The dye removal method on the Biomek FX will process a single 96-well plate of sequence reactions at a time with a final volume recovery of 25 μ L that can be directly loaded on the sequencer. The method has been optimized to purify a 10 μ l 1/20x BigDye Terminator 3.1 reactions. Steps such as washing, resuspension and vacuum times have been optimized to deliver consistent results and minimize contamination. The starting instrument setup for dye removal is shown below (see Fig. 4) A vacuum filtration manifold is affixed to the deck on the P7 site;

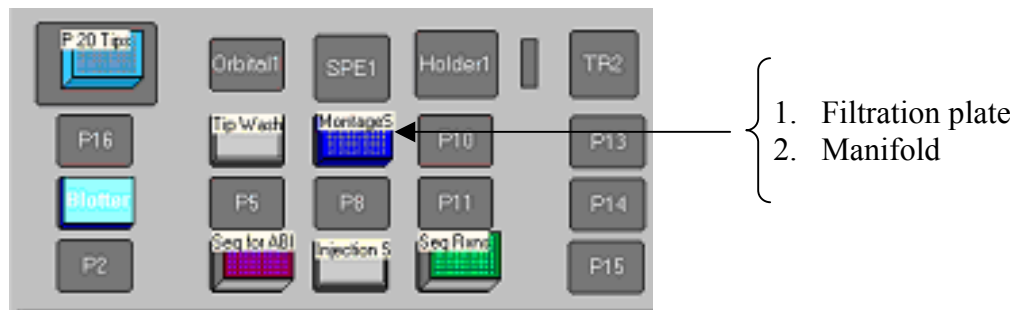


Figure 4 The starting instrument setup for dye removal method on Biomek FX

RESULTS AND DISCUSSION

Quantity and quality of cleaned sequences reactions obtained using the Biomek FX and Millipore Montage SEQ₉₆ Sequencing Reaction Cleanup Kit reagents was tested on the Applied Biosystems 3730 DNA Analyzer.

Capillary Sequencing

The suitability of the sequences reactions cleaned on the Biomek FX for capillary sequencing was verified on the Applied Biosystems 3730 DNA Analyzer. The array view of the sequences cleaned manually is shown in figure 5; the array view of the same sequence reactions cleaned on the Biomek FX with Millipore Montage SEQ₉₆ Sequencing Reaction Cleanup Kit reagents is shown in figure 6. Example of sequences from samples purified on Biomek FX with Millipore Montage SEQ₉₆ Sequencing Reaction Cleanup Kit reagents are shown in figure 7. The average read length obtained with the samples cleaned on Biomek FX on the Applied Biosystems 3730 DNA Analyzer was more than 800 base pairs. The accuracy of the sequence at 400 base pairs was excellent at approximately 99%.

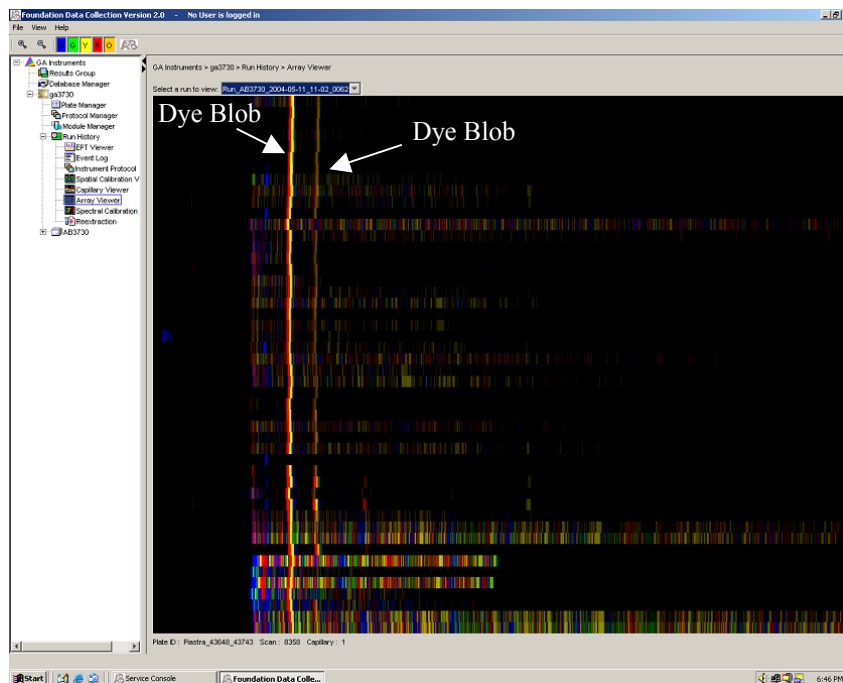


Figure 5 Applied Biosystems array view from plate purified manually as recommended

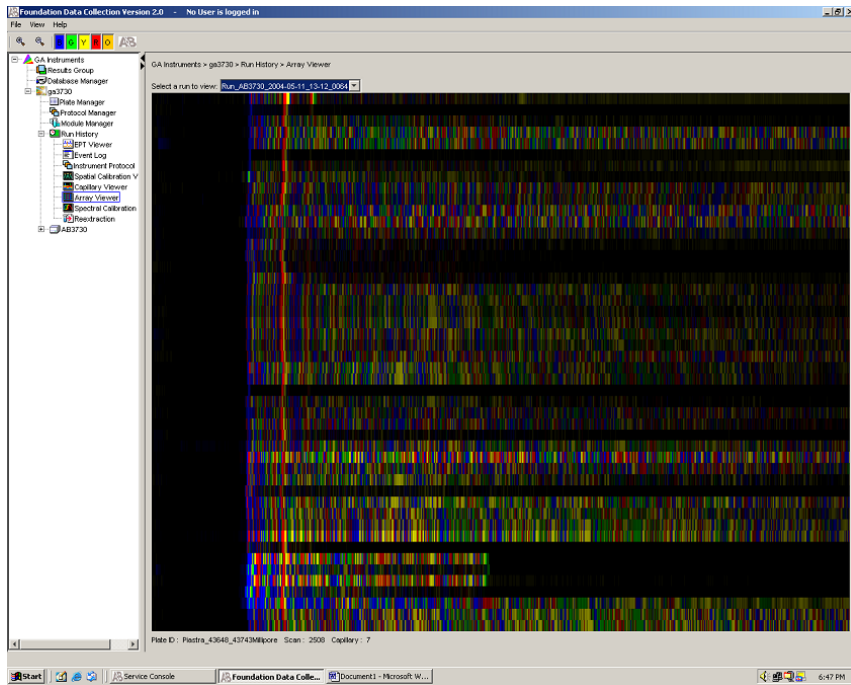


Figure 6 Applied Biosystems array view from plate purified on Biomek FX with Millipore Montage SEQ96 Sequencing Reaction Cleanup Kit reagents as described

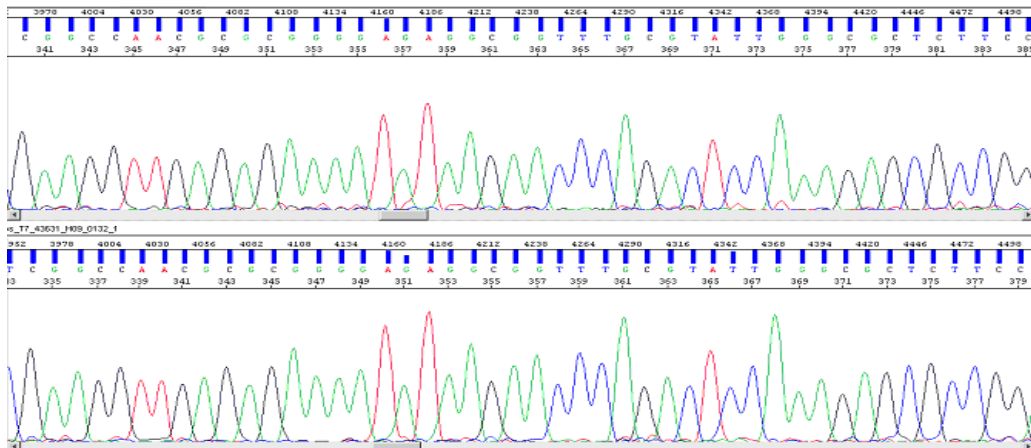


Figure 7 Sequencing data from purified pBluescript(+) sequenced on Applied Biosystems 3730 DNA Analyzer plasmid purified on Biomek FX.

CONCLUSIONS

The data presented here demonstrate that the Biomek FX Laboratory Automated Workstation, equipped with ORCA® robotic arm is a suitable platform for the high throughput dye removal of sequences reactions using Millipore Montage SEQ₉₆ Sequencing Reaction Cleanup Kit reagents.

The method and system described here:

- Generated good quality purified sequence reactions with a high signal return.
- Is able to manage high number of samples in complete, but flexible, automation and generate a complete samples tracking from the sources plates to the recovered samples.

Acknowledgments

We would like to thank D. Fiorentino, E. Oggioni and L. Carrara (Beckman Coulter Italy) for the great support to this job. The authors also would like to thank C. Raviolo, A. Marsiglia (Millipore Italy) for the precious suggestions and the material used for the tests.