MGE Newsletter N°18, January 27th 2006

High throughput plasmid purification using Millipore Montage Plasmid Miniprep Kit* and Beckman Coulter's Biomek® FX* Laboratory Automation Workstation equipped with ORCA® robotic arm

M. Borra, E. Mauriello, R. Pannone, E. Biffali.

Marine Genomics Europe (NoE) Technology platform # 22
SZN-Stazione Zoologica "A. Dohrn" Naples, It

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 preparation of plasmid DNA using Millipore Montage Plasmid Miniprep Kit. Using this system, plasmids are purified by vacuum filtration, using a unique separation technology that retain the DNA on a size-exclusion membrane while protein and contaminants are filtered trough the waste. Finally the DNA was recovered, by pipetting resuspension, from the membrane or stored in refrigerator using sealing tape for a following use. 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 purification of plasmids will be described:

• The automated Core System workstation: Biomek FX & ORCA
• The software and method to drive the workstation
• The results when purifying plasmids using Montage Plasmid Miniprep Kit

Representative data obtained from the purification of plasmids using this system will be shown. Data from capillary sequencing on Beckman Coulter's CEQ 2000XL DNA Analysis System and Applied Biosystems 3730 DNA Analyzer will be shown demonstrating the suitability of the purified plasmids for stringent assays such as capillary sequencing.

INTRODUCTION

Methodology for plasmid purification using Millipore Montage Plasmid Miniprep Kit reagents 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 of bacterial pellets at a time, until to a maximum of sixteen plates, to purified plasmids 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 38 minutes using only the liquid handler; using the complete core system (BFX, ORCA and Carousel) two 96-well plates can be processed in approximately 88 minutes, four in 177 minutes up to 16 plates in 711 minutes.

The results presented here demonstrate the quantity and quality of plasmid DNA generated using Millipore Montage Plasmid Miniprep Kit reagents on the Biomek FX. DNA quantity and quality were assessed by spectrophotometric analysis, gel electophoresys, PCR and sequencing analysis.

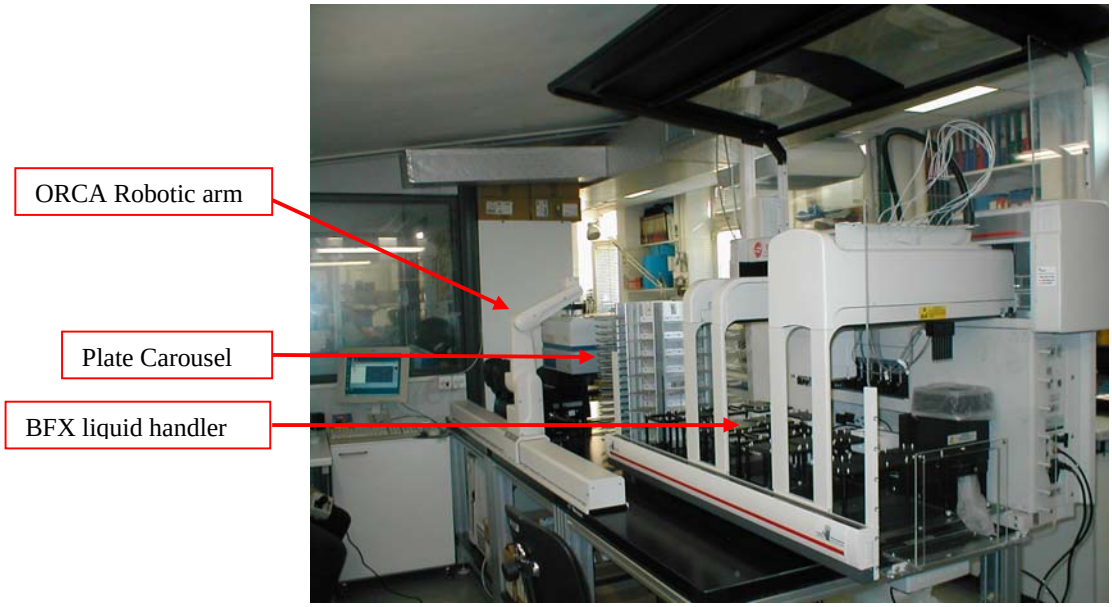

Figure 1 The automated Core System workstation: Biomek FX, plates carousel & ORCA

MATERIALS AND METHODS

Samples was grown in a 96-deep well culture block with1,2ml LB in an shaker incubator at 37°C, 225 rpm for at least 10 hours and then centrifugated. The supernatant was manually eliminated before starting the purification protocol.
Nucleic acids were purified on a Biomek FX dual bridge (see Figure 1). Hardware needed to purify plasmids on this platform includes a Gripper and a vacuum manifold. Used labware is removed from the worksurface of the instrument with ORCA. The collar and manifold have been designed to allow for elution into a standard microtiter plate. The Spacer Collar is used to stack the Clearing Plate and the Plasmid Plate (see Fig. 5). The first plate is used to remove cellular debris and the second to retain the DNA on a size-exclusion membrane while protein and contaminants are filtered trough the waste and the DNA was recovered by pipetting resuspension. The Gripper Tool provides for automated disassembly and reassembly of the stack (see Fig. 5).

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:

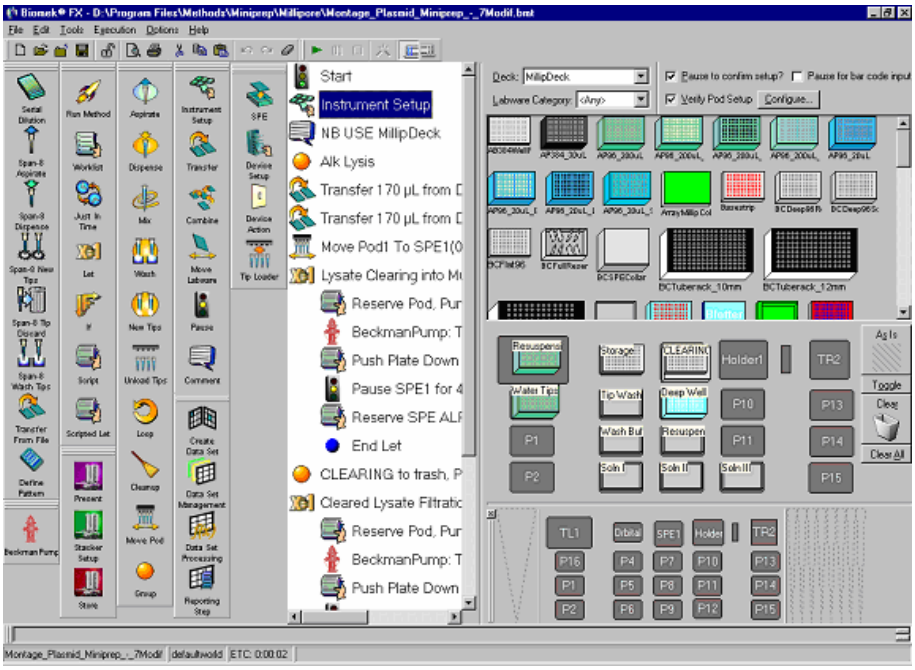

Figure 2 The plasmid purification method on the Biomek FX

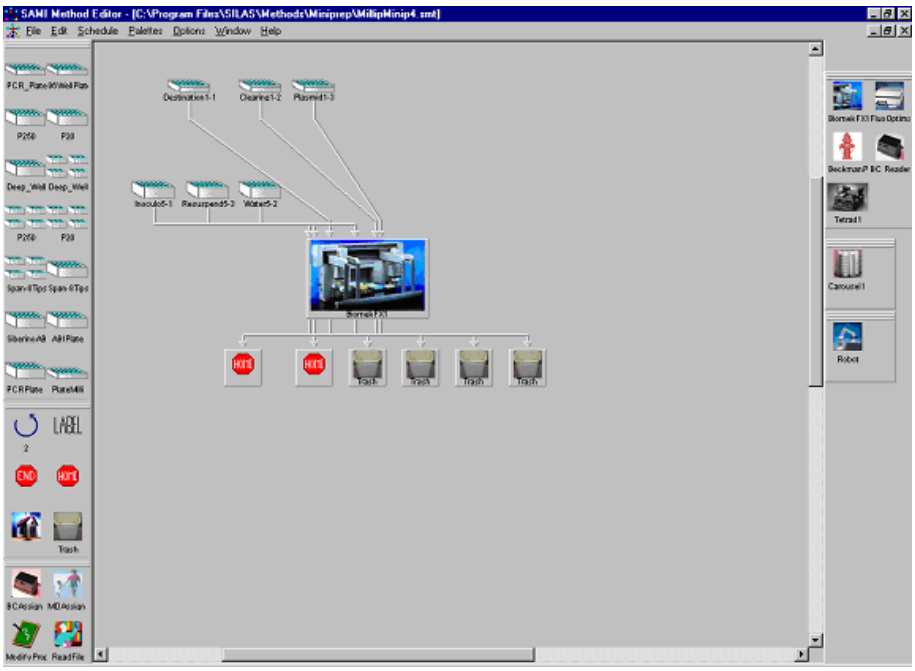

Figure 3 The plasmid purification method on SAMI software

The plasmid purification method on the Biomek FX will purify a single 96-well plate of bacterial pellets at a time with a final elution volume of 50µL. The method has been optimized to purify high quality plasmids. Steps such as bacterial pellet resuspension and vacuum times have been optimized to deliver consistent results and minimize contamination. The starting instrument setup for plasmid purification is shown below (see Fig. 4) A vacuum filtration manifold is affixed to the deck on the SPE site; using the gripper tool, the holder is accessed for the assembly and disassembly of the collar and plates (see Fig 5).

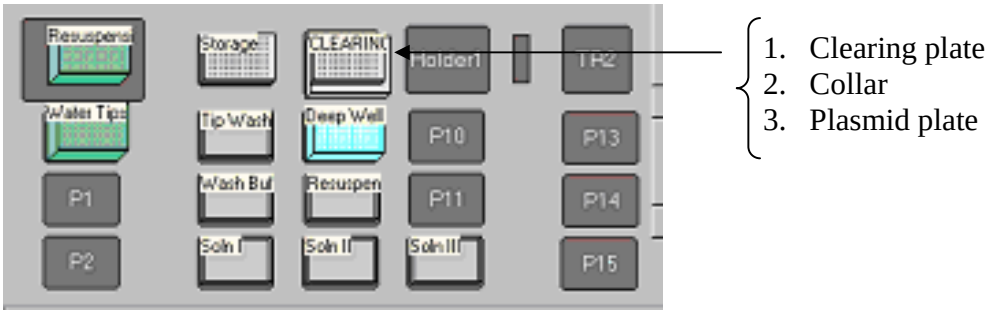

Figure 4 The starting instrument setup for plasmid purification method on Biomek FX

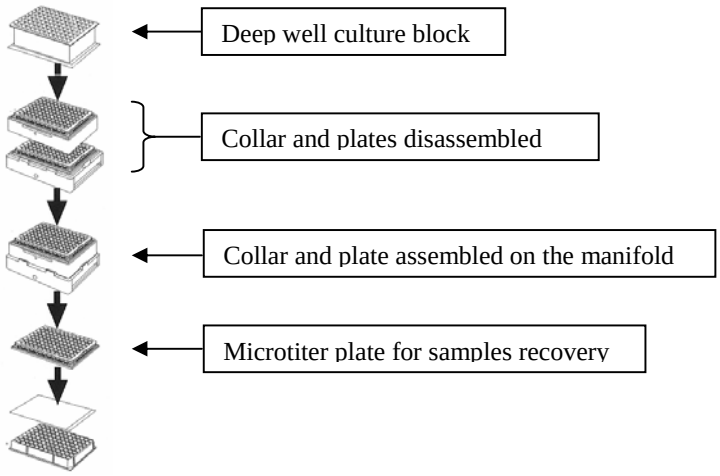

Figure 5 schematic view of the plates used in the protocol and the assembly scheme of collar and plates

RESULTS AND DISCUSSION

Quantity and quality of recovered plasmid DNA purified using the Biomek FX and Millipore Montage Plasmid Miniprep Kit reagents were determined by measuring absorbance at 260nm and 280nm, by gel electophoresys, PCR and sequencing analysis.

Spectrophotometric analysis

The spectrophotometric data, (data not shown) point out that purifying a high copy plasmid, pBluescript(+), with this method we can obtain, in a 50µL elution volume, an average concentration of ~40-80ng/µL of DNA. The purity of the cultures was consistently excellent as indicated by the OD260/280 ratio.

Gel electophoresys.

Quantity and quality of recovered plasmid DNA was tested by gel electophoresys. On a 1% agarose gel (TBA 0,5X with ethydium bromide) 5µl of recovered DNA was loaded using λII as marker. The result confirms the data obtained with spectrophotometric analysis. A gel image is shown below. (see Fig.6)

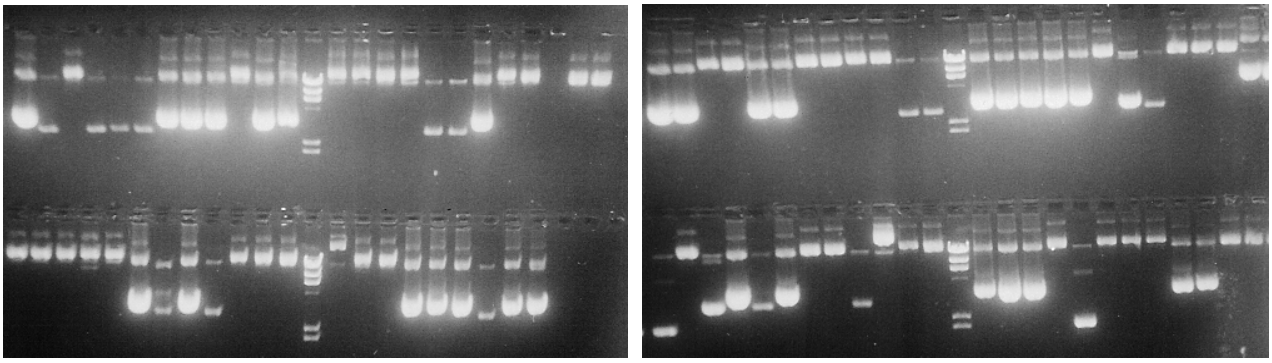

Figure 6 Gel electophoresys of plasmid purificated on Biomek FX

PCR Assay to Detect Cross Contamination

Bacterial culture was seeded into every other well such that cross-contamination could be assessed using a PCR-based assay. Eluted plasmids were used in an amplification reaction with commercially available M13 sequencing primers. Samples were easily amplified and no product was observed in wells without bacterial cells, indicating that contamination of adjacent wells did not occur during processing. Amplification products from samples processed on the Biomek FX are shown below. (see Fig 7)

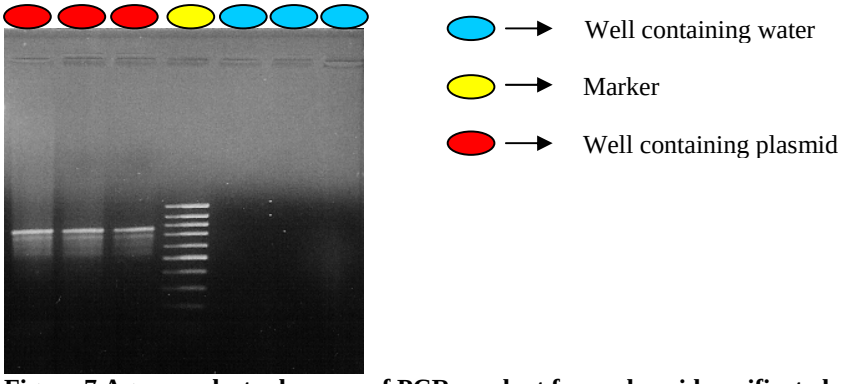

Figure 7 Agarose electophoresys of PCR product from plasmid purificated on Biomek FX

Capillary Sequencing

The suitability of plasmids purified on the Biomek FX for capillary sequencing was verified using Beckman Coulter's CEQ 2000XL DNA Analysis System and Applied Biosystems 3730 DNA Analyzer. Sequencing reactions and post-reaction cleanup were performed as recommended. Examples of sequence from a purified plasmid are shown below. (see Fig. 8)

The average read length obtained on the CEQ 2000XL DNA Analysis System was approximately 600 base pairs. The average read length obtained 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%.

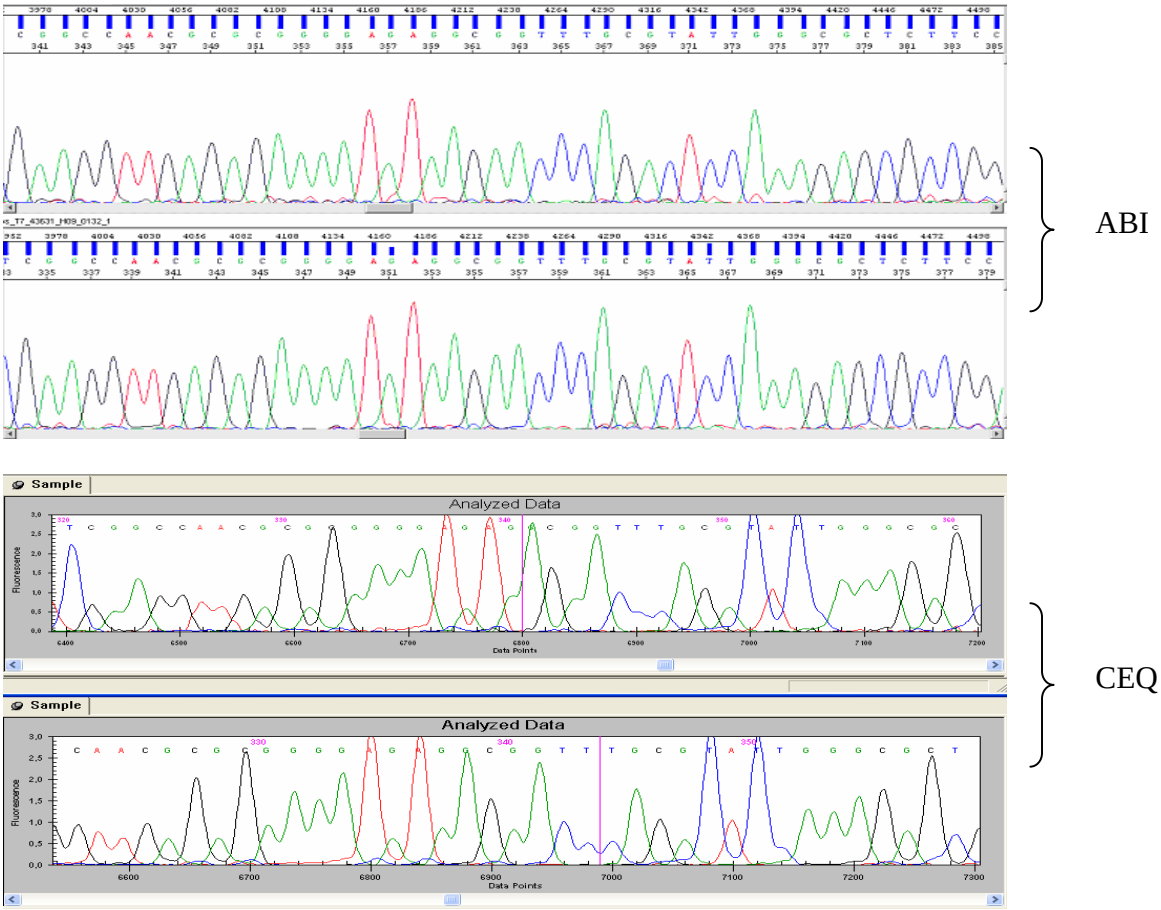

Figure 8 Sequencing data from purified pBluescript(+) sequenced on Beckman Coulter's CEQ 2000XL DNA Analysis System and Applied Biosystems 3730 DNA Analyzer plasmid purificated on Biomek FX.

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 purification of nucleic acids using Millipore Montage Plasmid Miniprep Kit reagents.
The method and system described here:

- Generated high quality plasmids from standard growth conditions using a high copy plasmid (pBluescript(+))
- Is able to recover a good quantity (enough for the tipical downstream applications like PCR, sequencing, etc.) of high quality DNA that can be directly sequenced after purification.
- 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.

Furthermore the introduced automated workstation is extremely flexible allowing us to minimize the costs and to adjust the protocols just “on-demands”, making the automated solution accessible for many different research groups.

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