Using Beckman Coulter's Biomek® NX Laboratory Automation Workstation for the Purification of High Quality Plasmid DNA

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ABSTRACT

The information included in this poster describes the utilization of a new automated liquid handler, the Biomek NX Laboratory Automation Workstation, in the preparation of plasmid DNA using Promega's Wizard* SV96 Plasmid DNA Purification System. A single plate of bacterial pellets can be purified on the deck of the Biomek NX in approximately 50 minutes. The following system components, for the purification of plasmids, will be described:

- The hardware requirements for the Biomek NX
- Software and method to drive the automation workstation
- Results when purifying plasmids using Wizard SV96 reagents.

Plasmids were analyzed by standard methodologies to assess the quality and quantity of product. Automated capillary sequencing was performed on Beckman Coulter's CEQ™ 8000 Genetic Analysis System thereby, demonstrating the suitability of the purified plasmids for capillary sequencing.

INTRODUCTION

Methodology for plasmid purification using Promega Wizard SV96 reagents was developed for the Biomek NX, a new, automated workstation from Beckman Coulter, Inc. (see Figure 1). This platform provides sufficient deck space to process one plate of bacterial pellets to purified plasmids without user interaction after initial setup. Furthermore, the Biomek® Software allows for variables to be included in the method thereby providing flexibility by having user-defined options such as the final elution volume. A 96-well plate can be processed in approximately 50 minutes, using the default settings.

The results presented here demonstrate the quantity and quality of plasmid DNA generated using Promega Wizard SV96 reagents on the Biomek NX. DNA quantity and quality for pGEM-3Zf(+) was assessed by spectrophotometric analysis, restriction digestion, PCR® and capillary sequencing. Additionally, plasmids isolated during a bacterial two-hybrid screening procedure were purified on the Biomek NX; the sequencing data obtained from these plasmids will be summarized.

® The PCR process is covered by patents owned by Roche Molecular Systems, Inc. and F. Hoffman La Roche, Ltd.

Materials and Methods

Biomek NX Hardware Configuration

Methods for the purification of nucleic acids were developed on a stand-alone, 96-Multichannel Biomek NX (Figure 1). The instrument uses the same Automated Labware Positioner (ALP) architecture as the Biomek FX. Therefore, static ALPs and active ALPs such as the Orbital Shaker ALP can be utilized on the deck of the Biomek NX.

Figure 1. The Biomek NX Laboratory Automation Workstation.

Biomek NX Deck Setup for Plasmid Purification

The deck used for plasmid purification is shown below. A vacuum filtration manifold is affixed to the deck on the SPE site. The holder ALP is used for the assembly and disassembly of the collar and filter plates.

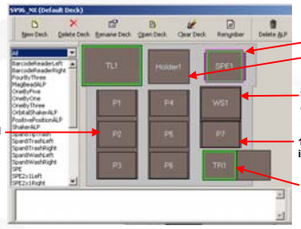

Figure 3. ALP positions on the deck of the Biomek NX.

Biomek NX Method

The plasmid purification method on the Biomek NX will purify a single 96-well plate of bacterial pellets. 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 Wizard SV96 plasmid purification is shown below.

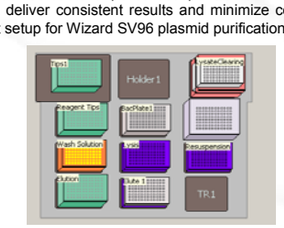

Figure 4. Starting instrument setup for plasmid purification on the Biomek NX.

Results

Plasmid Yield and Quality

The spectrophotometric data shown in Table 1 demonstrate the quantity and quality of pGEM-3Zf(+) plasmids using Wizard SV96 purification reagents on the Biomek NX. Overall yield was approximately 4 µg from a small biomass (~1.5 OD_{600}) with an average concentration of ~49 µg/mL using the default elution volume of 100 µL. The purity of the cultures was consistently excellent as indicated by the $OD_{260/280}$ ratio.

Plate	Bacterial Density (OD_{600})	Average Quality ($A_{260/280}$)	Average Yield (µg)
1	1.45	1.84+/- 0.19	3.82+/-1.13
2	1.45	2.04+/-0.58	3.83+/-1.44
3	1.45	1.88+/-0.26	4.03+/-0.98
AVG		1.92+/- 0.34	3.89+/-1.18

Table 1. Quantity and quality of pGEM-3Zf(+) purified using the Biomek NX Wizard SV96 method. Yield and purity were determined by measuring absorbance at 260nm and 280nm.

PCR Amplification of Purified Plasmids

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 custom primers designed to generate a 752 base pair fragment. 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.

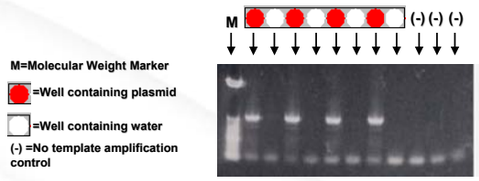

Figure 6. Agarose electrophoresis of PCR products from pGEM-3Zf(+) plasmids. Sample order as indicated.

Capillary Sequencing on Beckman Coulter's CEQ 8000 Genetic Analysis System

The suitability of plasmids purified on the Biomek NX for capillary sequencing was verified using Beckman Coulter's CEQ 8000 Genetic Analysis System. Sequencing reactions and post-reaction cleanup were performed as recommended in the standard protocol. Typical sequencing results obtained are shown below.

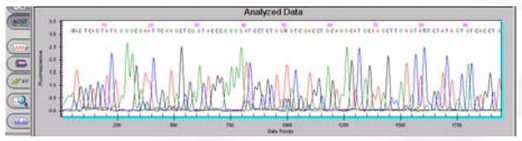

Figure 7. Sequencing data from purified pGEM-3Zf(+) sequenced on Beckman Coulter's CEQ 8000 Genetic Analysis System and analyzed with the analysis module.

Sequencing Protocol for Plasmids Isolated in Two-Hybrid Experiment

The eluted plasmids were used directly in a sequencing reaction with CEQ Dye Terminator Cycle Sequencing reagents as follows:

- 6 µL plasmid, 1.6 µM custom primer to the vector, and 12 µL DTCS premix were aliquotted into thermo well plates.
- After an initial denaturation for 2 minutes at 98°C, the reaction was amplified using 30 cycles of 96°C for 20 seconds, 50°C for 20 seconds, and 60°C for 4 minutes followed by holding at 4°C.
- The amplified products were loaded onto a CEQ 8000 Genetic Analysis System.

Capillary Sequencing of Two-Hybrid Clones on Beckman Coulter's CEQ 8000 Genetic Analysis System

Representative sequence generated from one of the two-hybrid clones is shown below. Importantly, good quality sequence could be obtained with minimal changes to the methodology to accommodate purifying a low copy plasmid in an endonuclease-positive strain of bacteria.

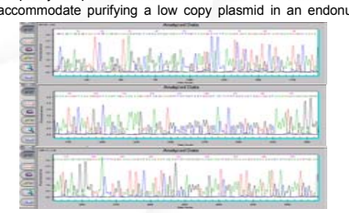

Figure 8. Sequencing data from a purified two-hybrid clone sequenced on Beckman Coulter's CEQ 8000 Genetic Analysis System and analyzed with the analysis module.

Software Used on the Biomek NX

Biomek Software is used to control the Biomek NX. It is a common software architecture among Beckman Coulter's Laboratory Automation Workstations (Biomek NX, the Biomek 3000 and the Biomek FX). Software functionality includes features such as variables, version control, and sample tracking. A view of the plasmid method is shown below.

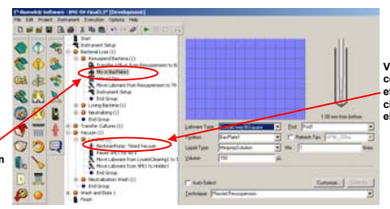

Figure 2. The plasmid preparation method on the Biomek NX.

Restriction Digestion of Purified Plasmids

After purifying pGEM-3Zf(+) using the Biomek NX single-plate method, restriction digests were performed to show the suitability of the eluted plasmids for typical downstream experiments. Three different enzymes were used in the analysis (Figure 5).

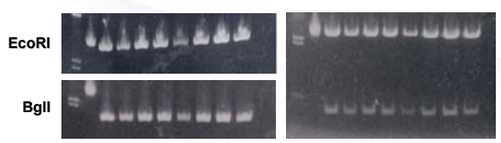

Figure 5. pGEM-3Zf(+) purified with Wizard SV96 reagents on the Biomek NX and digested with various enzymes as indicated. Lane order for all gels is: Lane 1=Markers, Lane 2=uncut plasmid, Lanes 3-10=digested plasmids.

Sequencing Data for Plasmids Purified on the Biomek NX

Sequencing data from DNA isolated on the Biomek NX are summarized in the table below. The average read length obtained on the CEQ 8000 Genetic Analysis System was approximately 695 base pairs. The accuracy of the sequence data at 600 base pairs was excellent at approximately 98%.

Plate	Accuracy at 600 base pairs	Read Length in base pair	Samples
1	99.2+/-0.007	702.3+/-58	n=16
2	95.2+/-0.12	679.7+/-129	n=16
3	99.6+/-0.002	702.9+/-40	n=16
Average	**98.0+/-0.043**	**694.9+/-75**	**n=48**

Table 2. Sequencing summary of pGEM-3Zf(+) samples purified on the Biomek NX using the single-plate method.

Bacterial Two-Hybrid Experiment

To show the utility of the method and platform for perfoming experimentation beyond purifying high copy number plasmids such as pGEM-3Zf(+), bacterial colonies isolated during a bacterial two-hybrid screen# were purified using the plasmid purification method for the Biomek NX. The following parameters were changed to accommodate the low copy plasmid in an endonuclease positive strain of *E. coli*:

- Alkaline protease was added to the lysis buffer in a ratio of 40 µL alkaline protease per mL lysis buffer.
- The vacuum time for clearing lysates was increased to 10 minutes.
- The elution volume was decreased to 60 µL.

Purified plasmids from 136 clones were used directly in a sequencing reaction.

#This work was done in collaboration with Loren J. Field, Ph.D., Professor of Medicine, Indiana University School of Medicine, Indianapolis,Indiana.

Summarized Sequencing Results for Plasmids Isolated in Two-Hybrid Experiment

From the 136 samples purified and sequenced, results were obtained from GenBank searches with the following definitions:

- Significant homology=majority of sequence of an isolate has homology to sequences in GenBank
- Limited homology=isolated regions of homology to sequences in GenBank
- No significant homology=no homology or less than 25 base pairs of homology to sequences in GenBank

	Number of samples
Significant Homology	112
Limited Homology	14
No Significant Homology	10
TOTAL	136

Table 3. Sequencing summary of bacterial two-hybrid samples purified on the Biomek NX using the single-plate method.

CONCLUSION

The data presented here demonstrate that the Biomek NX can be utilized in molecular biological processes such as the purification of plasmids. The method and system described here:

- Generated high quality plasmids from standard growth conditions including using a high copy plasmid (pGEM-3Zf(+)) and an endonuclease negative strain of bacteria (DH5α).
- Produced plasmids from colonies isolated in a bacterial two-hybrid screening experiment that were directly sequenced after purification.

The methodology on the Biomek NX only required slight modifications to accommodate the low copy number plasmid and the endonuclease positive strain of bacteria.

Acknowledgments

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