Wizard® SV 96 Plasmid DNA Purification System: High Quality Plasmid DNA for Use in Fluorescent Sequencing Methods

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Wizard® SV 96 Plasmid DNA Purification System[a] is a complete system designed for the rapid purification of up to 96 plasmid DNA samples in about 1 hour. The system may be used either manually or with automated robotics (e.g., the Biomek® 2000 Workstation) to yield highly pure DNA suitable for automated fluorescent sequencing, restriction enzyme digestion and other molecular biology applications. Plasmid DNA purified using the Wizard® SV 96 System is compatible with Dye Primer, BigDye™ Primer and BigDye™ Terminator sequencing chemistries.

Introduction

The Wizard® SV 96 Plasmid DNA Purification System provides a simple method for the rapid isolation of high quality plasmid DNA from as many as 96 samples at a time (Figure 1). The entire procedure can be completed in under one hour using the Vac-Man® 96 Vacuum Manifold in manual format. The Wizard® SV 96 System can also be used with several automated platforms, such as the BioRobot 9600 (Qiagen) and the Biomek® 2000 Laboratory Automation Workstation (Beckman Coulter, Inc.). Using automated systems, the time from pelleted cells to eluted DNA is approximately 65 minutes. The purified plasmid DNA can be used directly following elution for automated fluorescent sequencing as well as other molecular biology applications, such as restriction enzyme digestion. In this report we demonstrate the superior design and performance of the Wizard® SV 96 Plasmid DNA Purification System, both manually and on the Biomek® 2000 Workstation, for isolating high quality plasmid DNA for automated sequencing.

Convenient and Flexible Design

The Wizard® SV 96 Plates have a number of design improvements to reduce trapping wash effluent at the well tips. Unlike other commercially available 96 well plates, there is no skirt around the tip, and the diameter of the tip aperture is increased to eliminate spraying of flow through (Figure 2).

The Vac-Man® 96 Vacuum Manifold was designed for ease of use in processing 96 samples in the manual format and to be adaptable to automation platforms of existing liquid handling instruments. The manifold is small (5.75 × 4 inches) taking up minimum space on the deck of automated instruments. The liquid waste goes directly to a waste trap without accumulating in a waste tray in the base of the manifold. This eliminates the need to disassemble the manifold during the process to discard wash solution. The manifold configurations for lysate clearing and DNA binding and elution of purified DNA are shown in Figure 1.

Automated Plasmid DNA Purification on the Biomek® 2000 Workstation

Although the protocol for the automated Wizard® SV 96 Plasmid DNA Purification System with the Biomek® 2000 is similar to the manual procedure, several changes were made to accommodate the limitations of and incorporate the advantages of purification using the robot. The important changes are that the volumes of cell resuspension, lysis and neutralization solution have been cut in half. The lowered volume requirements for resuspension, lysis and neutralization solutions eliminate steps and save time and reagents, resulting in a quick, easy transition from lysate clearing to DNA binding and elution. Optimal performance of the Wizard® SV 96 System is achieved when it is used with Promega's Vac-Man® 96 Vacuum Manifold and an additional manifold collar (available separately, Cat.# A2311) on the Biomek® 2000 Workstation. The use of the additional manifold collar increases efficiency by eliminating several movements of the robot arm.

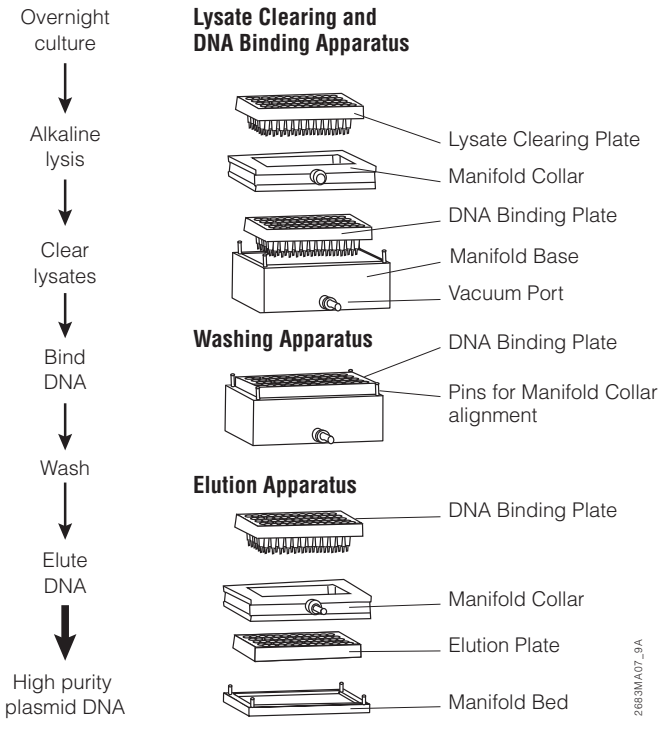

Figure 1. Protocol overview. Flow diagram of plasmid DNA isolation and purification using the Wizard® SV 96 Plasmid DNA Purification System. See Figure 2 for close-up view of the enhanced tip design of the Wizard® SV 96 Plate.

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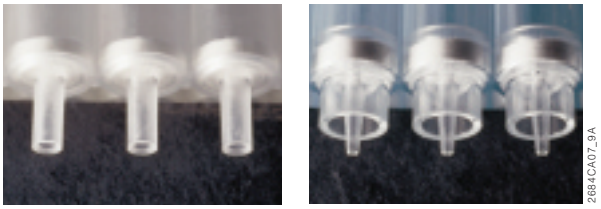

Figure 2. Plate tip design comparison. Photographs show increased tip aperture and absence of skirt for the Wizard® SV 96 Plate tips (left) versus the tips of a second commercially available 96 well plate (right).

All reagent additions are handled by the MP 200 8-tip dispensing tool on the Biomek® 2000. Reagent reservoirs, pipette tips, assay plates and the Vac-Man® 96 Vacuum Manifold are positioned on the robot. Movement of labware and disposal of spent plates are all performed by the Biomek® 2000 gripper arm, allowing start-to-finish plasmid preparation without assistance by the researcher. Purification takes only 65 minutes for automated processing of 96 miniprep plasmid DNA samples.

Growth Conditions for Plasmid-Containing Strains of *E. coli*

Optimal conditions for growing *E. coli* host strains containing plasmids are highly variable. Biomass and plasmid DNA yield are dependent on the host/vector combination used as well as a myriad of other factors. (*Biomass* is discussed in the article immediately following.) The culture conditions suggested here are based on experience gained during the development and testing of the Wizard® SV 96 System, using a high copy number plasmid (pGEM®-3Zf(+) Vector[b]) and DH5α® bacterial cells. Growth media containing the appropriate antibiotic can be dispensed into each well of a deep well culture plate. If greater than 6µg of DNA per miniprep sample is needed, we recommend Terrific Broth or CIRCLEGROW® media. We do not recommend the use of 2X YT culture medium; restriction digestion of plasmid DNA purified from bacterial cultures grown in 2X YT can be problematic. The data reported here were obtained using cells grown in Terrific Broth medium. Studies using 1.0ml of DH5α® cells and pGEM®-3Zf(+) Vector grown in Terrific Broth medium with ampicillin have typically yielded 8–12µg of plasmid DNA.

Yield and Purity of Plasmid DNA

The Wizard® SV 96 System has been tested extensively for consistency of yield and purity. Table 1 shows purity and yield data for 3 × 96 well plates processed using the Biomek® 2000 Workstation. Cells were grown to a density of 40 A_{600} units before processing. Sixteen samples per plate (48 samples total) were analyzed. Average DNA yields for cell samples were 12.5µg. The average A_{260}/A_{280} ratio, which indicates purity of the isolated DNA, was 1.88. Similar results were obtained for plasmid DNA when the Wizard® SV 96 System was used in conjunction with the BioRobot 9600 (data not shown).

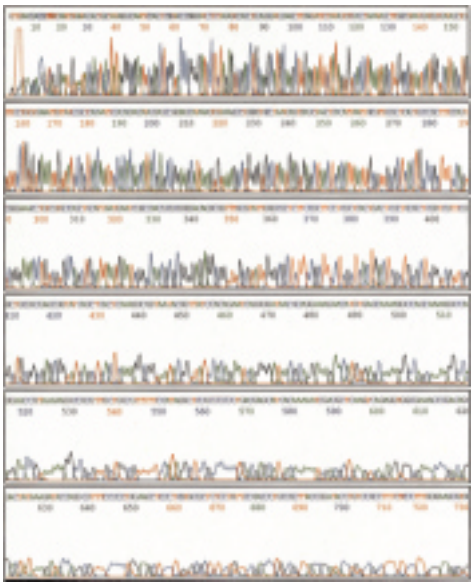

Figure 3. Electropherogram of DNA sequence following isolation by the Wizard® SV 96 System and cycle sequencing using BigDye™ Terminator reactions. Results demonstrate >700 consecutive bases analyzed with >98% accuracy of base identity.

Automated DNA Sequencing

The Wizard® SV 96 Plasmid DNA Purification System was designed to yield plasmid DNA for use as a template in DNA sequencing reactions. The BigDye™ terminator cycle sequencing kit (PE Applied Biosystems) was developed specifically for fluorescent DNA sequencing. Our performance specification for a 600 base read using BigDye™ terminator is >98% DNA sequence accuracy for the Wizard® SV 96 System. DNA samples prepared with the Wizard® SV 96 System in manual and automated formats were tested to verify the sequencing specification using ABI PRISM® cycle sequencing ready reaction kit.

Table 1. Yield and Purity of DNA Isolated with the Wizard® SV 96 System.

Plate	Yield (µg)	Purity (A_{260}/A_{280})
1	10.5 ± 1.3	1.90 ± 0.06
2	13.1 ± 2.4	1.89 ± 0.03
3	12.8 ± 1.1	1.89 ± 0.05

Data are averages of 16 samples per plate.

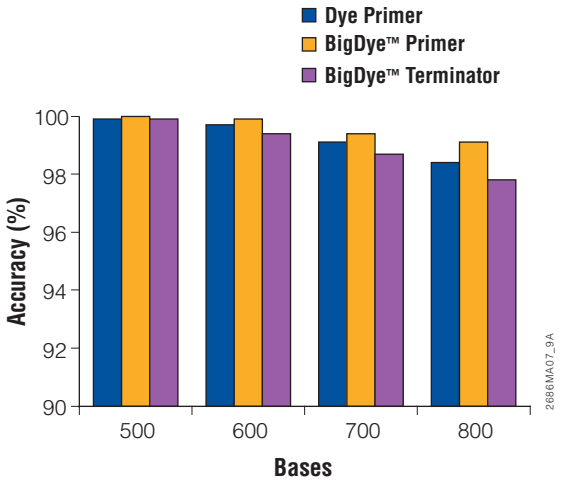

Figure 4. Accuracy by read length for plasmid DNA sequenced following isolation by the Wizard® SV 96 System and cycle sequencing using Dye Primer, BigDye™ Primer and BigDye™ Terminator kits. DNA direct from the Wizard® SV 96 Elution Plate was used with each of the three sequencing reactions using reaction conditions and cycling parameters as specified by the manufacturers (1–3).

BigDye™ Terminator: The BigDye™ terminator reaction included 0.5µg template DNA and 3.2pmol primer (pUC/M13 Forward Primer, Promega Cat.# Q5391), 8µl BigDye™ terminator reaction mix diluted 1:2 (in 1mM $MgCl_2$, 10mM Tris-HCl [pH 9.0]) (PE/ABI Cat.# 4303149) in a final volume of 20µl sterile water. A GeneAmp® PCR System 9600 was used for cycle sequencing and results were analyzed on an ABI PRISM® 377 (Perkin-Elmer) machine. Sequencing reactions were prepared and loaded as noted in reference 2, 1.5µl reaction mix per lane on a 4% acrylamide/6M urea gel. **Note:** For direct sequencing, the estimated yield of plasmid DNA must be ≥3µg to ensure a DNA concentration of >0.05µg/µl (in 65µl eluate). The maximum recommended template volume for BigDye™ terminator templates is 10µl.

BigDye™ Primer: The BigDye™ Primer reaction included 0.4µg template DNA per A-G-C-T reaction set. Cycle sequencing parameters were as specified (1) with an added ramp to 96°C for two minutes before cycling.

Dye Primer: The Dye Primer reaction included 1.2µg of DNA template per A-G-C-T reaction set. The DNA was prepared by drying under vacuum, then suspending in water to a final concentration of 0.2µg/µl in 10µl.

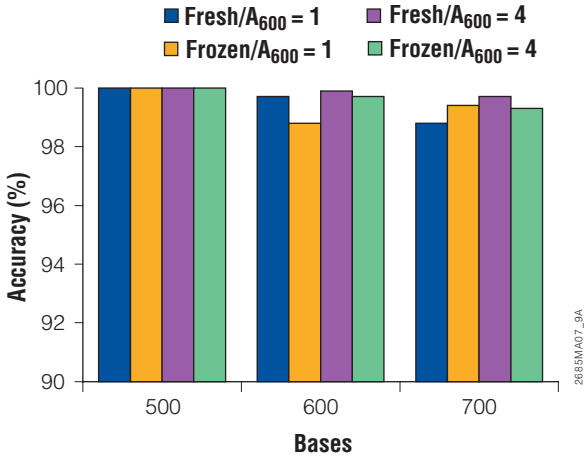

Figure 5. Accuracy and read length of plasmid DNA from fresh and frozen cultures of DH5α® cells grown to densities of 1 and 4 (A_{600}) units. Plasmid DNA was sequenced using BigDye™ terminator reactions as indicated in Figure 4.

When the BigDye™ terminator kit was used, 0.5µg plasmid DNA template was added directly from the elution plate with no additional concentration step. A representative electropherogram of BigDye™ terminator results using Wizard® SV 96 System-purified DNA is shown in Figure 3. As evident in Figure 3, over 725 bases were read with no errors.

Figure 4 compares automated sequencing results for Wizard® SV 96 System-purified DNA using three commercially available automated sequencing systems, BigDye™ terminator, BigDye™ primer and Dye Primer cycle sequencing kits. BigDye™ terminator sequencing was performed as noted above for Figure 3.

For BigDye™ primer sequencing, the template consisted of 0.4µg plasmid DNA per A-G-C-T reaction set. A reaction volume of 5µl was used as recommended for 1X reactions (1). Template DNA was used directly from the elution plate. The sequencing reactions were prepared as described (2). Samples (1.5µl) were loaded into each lane of a 4% acrylamide/6M urea gel.

With Dye Primer sequencing chemistry, 1.2µg of plasmid DNA template was used per A-G-C-T reaction set (3). The DNA was prepared by drying under vacuum then resuspending in water to a final concentration of 0.2µg/µl. The reaction volume was 10µl. The electrophoresis was performed as described above for BigDye™ terminator and primer systems.

The results obtained using these three methods are summarized in Figure 4. The Wizard® SV 96 System prepares DNA samples that can be sequenced >700 bases from the oligonucleotide primer with greater than 98% accuracy for all chemistries and formats used without DNA concentration post elution. It is noteworthy that the dye reagent in the reaction mix can be diluted by 50% without negative consequences to the read length and sequence accuracy.

Sequencing of Fresh vs. Frozen Plasmid DNA Samples

Since the Wizard® SV 96 System product specification for sequencing is based on BigDye™ terminator chemistry, several conditions for plasmid DNA template preparation were tested. DNA was prepared from fresh and frozen cells and 1 and 4 A_{600} were processed per well. The DNA samples were prepared on the Biomek® 2000 and the dye reagent was diluted 1:2 for all reactions and sequenced.

Plasmid DNA isolated from both fresh and frozen host cells gave comparable sequencing results in terms of signal intensity and base read accuracy. The results also indicated that the plasmid DNA concentration in the final elution from cells with A_{600} values of 1 and 4 were of sufficient concentration after purification that they could be used directly in the sequencing reaction (Figure 5).

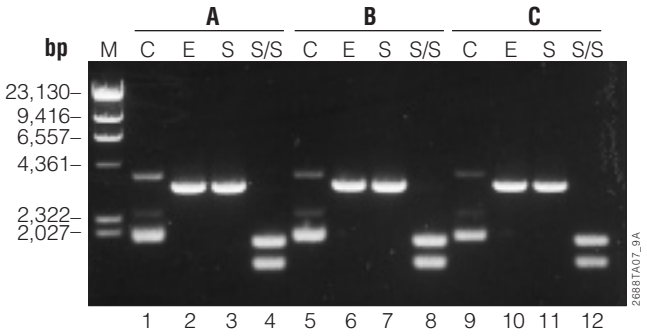

Figure 6. Analysis of restriction enzyme digestion of plasmid DNA in DH5α® cells. Lane M, Lambda DNA/*Hin*d III Markers (Cat.# G1711). Negative control (C, lanes 1, 5 and 9); *Eco*R I (E, lanes 2, 6 and 10); *Sca* I (S, lanes 3, 7 and 11); *Sca* I/*Sph* I (S/S, lanes 4, 8 and 12). The negative control reaction lanes show expected supercoiled, relaxed and nicked species of plasmid DNA, lanes E and S show linearized plasmid and lanes S/S show the two expected fragments. Restriction digestions were performed on three different plasmid DNA preparations (A–C). All digestion reactions included 10 units of enzyme per microgram of plasmid DNA (pGEM®-3Zf(+) Vector). The reactions were incubated for one hour at 37°C. All plasmid DNA was purified by Wizard® SV 96 Plasmid DNA Purification System from DH5α® cells with 4 A_{600} units.

Restriction Enzyme Digestions

One specification of the Wizard® SV 96 Plasmid DNA Purification System calls for the complete digestion of 1µg plasmid DNA by 10 units of restriction enzyme in 1 hour (at the appropriate temperature). As evident in Figure 6, plasmid DNA prepared using Wizard® SV 96 System was restricted completely by *Eco*R I, *Sca* I and *Sph* I including double digestions by *Sca* I/*Sph* I. Reaction conditions are defined in Figure 6.

Conclusion

The Wizard® SV 96 Plasmid DNA Purification System has been shown to yield high quality DNA at concentrations ready for DNA sequencing reactions used in ABI® Dye Primer, BigDye™ Primer and BigDye™ Terminator sequencing protocols. The 96 well format allows manual processing of 96 samples from pelleted cells to purified DNA template in approximately 60 minutes using the Vac-Man® 96 Manifold. The DNA sample preparation process can be adapted to several automated platforms and has been verified and validated on the Beckman BioMek® 2000. The quality of DNA purified is equivalent in both manual and automated formats. In addition, DNA purified from fresh and frozen cells is of similar quality. The ABI® Dye Primer protocol routinely gave 700 base reads with 99% accuracy and 800 base reads with 98% accuracy. The BigDye™ Primer protocol gave 800 base reads with over 98% accuracy. The BigDye™ Terminator protocols gave 600 base reads with greater than 99% accuracy and 700 base reads with 99% accuracy. The BigDye™ can be diluted two-fold in the sequencing reactions with no loss of read length or percent accuracy.

References

1. *ABI PRISM® BigDye™ Primer Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA Polymerase, FS Protocol.* P/N 403057 Rev. B. (1997).
2. *ABI PRISM® BigDye™ Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA Polymerase, FS Protocol.* P/N 4303237 Rev. B. (1998).
3. *ABI PRISM® Dye Primer Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA Polymerase, FS Protocol.* P/N 402113 Rev. B. August, 1995.


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Ordering Information

Product	Size	Cat.#	Price ($)
Wizard® SV 96 Plasmid DNA Purification System	1 × 96 preps	A2250	185
	5 × 96 preps	A2255	710
Vac-Man® 96 Vacuum Manifold	each	A2291	250
Vac-Man® 96 Vacuum Manifold Collar	each	A2311	65

[a]Patent Pending

[b]U.S. Pat. No. 4,766,072.