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Automated Plasmid Purification Using MagneSil™ Paramagnetic Particles

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Abstract

The Wizard® MagneSil™ Plasmid Purification System provides a reliable high-throughput method for plasmid DNA purification in a 96 well format. Here we present protocols for processing multiple plates of DNA samples with no manual intervention for multiple robotic platforms. Plasmid DNA yields of 6–9µg are routinely obtained. Purified DNA is ready-to-use in automated fluorescent sequencing.

The Wizard® MagneSil™ Plasmid Purification System provides a simple and reliable method for the rapid isolation of plasmid DNA in a 96 well format.

Introduction

High-throughput DNA sequencing demands fully automated, low-cost, robust sample preparations of purified plasmid DNA. Robotic protocols adapting conventional purification methods rely on vacuum filtration for the removal of debris from cell lysate and plasmid DNA binding. Instead, Promega's MagneSil™ systems use paramagnetic particles (PMPs) for DNA purification.

The use of PMPs for lysate clearing, as well as DNA capture, circumvents the need for centrifugation or vacuum manifolds, making the system ideal for full automation. Multiple plates can be processed on an automated platform with a robot arm without manual intervention. The Wizard® MagneSil™ Plasmid Purification System provides a simple and reliable method for the rapid isolation of plasmid DNA in a 96 well format. The purified plasmid can be used directly for automated fluorescent DNA sequencing as well as other molecular biology techniques. MagneSil™ Paramagnetic Particles^(a) (PMPs) are scalable, allowing nucleic acid capture methods to be adjusted for higher yield or for use in a 384 well format.

We provide information and recommendations for maximizing yield, bacterial growth conditions, and obtaining consistent recovery, all of which lead to highly accurate fluorescent sequencing results.

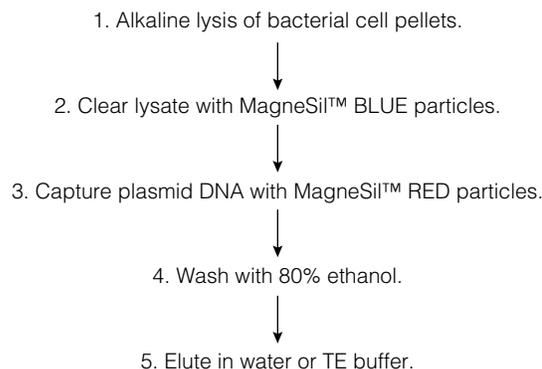


Figure 1. The Wizard® MagneSil™ Plasmid Purification System procedure is accomplished in five basic steps. All steps of the process are performed in standard 96 well microwell plates. Particle capture is performed using the MagnaBot® 96 Magnetic Separation Device (Cat.# V8151).

Purifying Plasmid DNA

MagneSil™ PMPs are used throughout the plasmid purification process. MagneSil™ BLUE^(a) particles are added to bacterial lysates to effectively clear cell debris; these particles do not bind nucleic acid, and plasmid DNA remains in the supernatant. MagneSil™ RED^(a) particles capture plasmid DNA. The procedure is accomplished in five basic steps (Figure 1).

All steps of the process are performed in a standard 96 well Microtiter® plate. The MagnaBot® 96 Magnetic Separation Device (Cat.# V8151), adaptable to most robotic platforms, captures the particles. Twenty-four powerful magnetic pins attract the particles to the sides of four wells in seconds, allowing easy removal of the particle-free fluid. A detailed protocol is available in the Wizard® MagneSil™ Plasmid Purification Technical Bulletin #TB286, available online at: www.promega.com/tbs/tb286/tb286.html.

Growing Bacterial Cells

Plasmid yield and quality can be influenced by multiple factors such as growth conditions, plasmid copy number and host strain. We recommend CircleGrow® Media (QBiogene), although Terrific Broth and Luria Broth may also be used depending on the growth characteristics of the host strain. As shown in Figure 2, cell densities up to

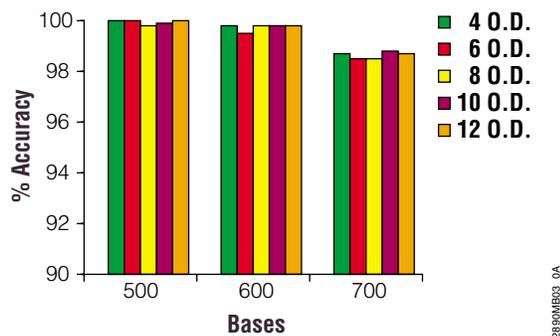


Figure 2. Accuracy by read length for plasmid DNA. Samples were sequenced following isolation by MagneSil™ Paramagnetic Particles. Cells were grown to the indicated amount of biomass (O.D.₆₀₀). Samples were purified and sequenced using BigDye™ Terminator cycle sequencing on the ABI PRISM® 3700 DNA analyzer and 377 DNA sequencer.

12 O.D.₆₀₀ can be processed using this protocol. Measurements of plasmid yield versus culture density indicate that plasmid recovery tends to plateau above 8.0 O.D.₆₀₀, due in part to limitations in volumes of cell lysis reagents. The optimal range of biomass (O.D.₆₀₀) as measured by turbidity for this protocol is 1.0–6.0. It is important to note that the biomass produced is dependent upon the culture medium, growth time and temperature, agitation speed, host strain, plasmid copy number, and the insert. For applications such as fluorescent DNA sequencing, special consideration should be given to the selection of plasmid and *E. coli* strains to optimize yield and quality of plasmid DNA. Optimal automated fluorescent sequencing results are obtained by using high-copy plasmids and *endA*⁻ bacterial strains.

Use a Shaker to Maximize Plasmid Yield

Correct mixing of MagneSil™ particles and cell lysates is critical for obtaining good yields of plasmid DNA. We have found that plasmid recovery is maximized when mixing is performed using a microtiter plate shaker. Moreover, a shaker minimizes variability in yield across samples and maximizes protocol efficiency compared to mixing by pipetting. An orbital shaker such as the Micromix® 5 (DPC, Inc.) can be integrated with a number of automated platforms including the Biomek® 2000 (Beckman Coulter). For more information on automated protocols and equipment requirements, please see: www.promega.com/nap/magnesil/.

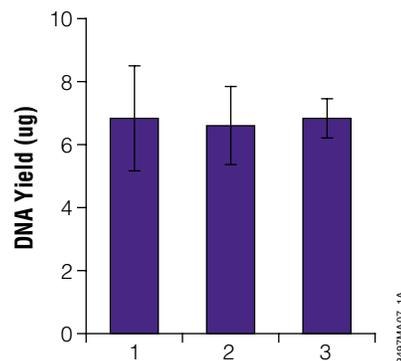


Figure 3. Consistent yield of plasmid DNA. Samples were purified using MagneSil™ Particles on a Biomek® FX workstation. Data are presented as average yield (µg/well) for three separate 96 well plates.

Obtain Consistent Recovery

Using the MagneSil™ plasmid purification protocol, one can efficiently process a broad range of culture biomass (1.0–6.0 O.D.₆₀₀) per sample well. As demonstrated in Figure 3, the yield ranges from 6–9µg of plasmid DNA at a concentration range of 60–100ng/µl from cells grown to a density of 4.0 O.D.₆₀₀. These results are well within the recommended range (200–500ng/reaction) for automated cycle sequencing of double-stranded plasmid DNA.

A detailed protocol is available in the Wizard® MagneSil™ Plasmid Purification Technical Bulletin #TB286, available online at www.promega.com/tbs/tb286/tb286.html

Generate High Purity Plasmid DNA for Fluorescent Sequencing

Plasmid DNA prepared using MagneSil™ PMPs has been rigorously tested to ensure optimal performance as templates in fluorescent BigDye™ Terminator cycle sequencing on the ABI PRISM® 3700 DNA analyzer and 377 DNA sequencer. As evident in Figure 4, all samples yielded DNA that provided 600+ base reads with >98% accuracy, as analyzed by the ABI base-calling program.

Samples analyzed using the Wizard® MagneSil™ Plasmid Purification System consistently give a score of 20 or greater when evaluated by Phred analysis. The Phred score is a quality value that indicates the probability that the base call is correct. A score of 20 indicates a 99% chance that the base call is correct.

Automated Plasmid Purification...continued

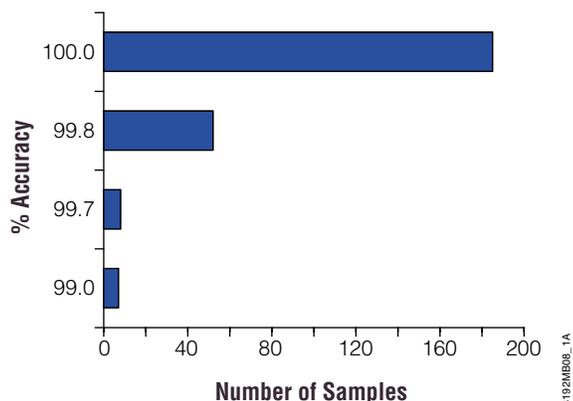


Figure 4. Reproducibility of accuracy in read length. Plasmid DNA samples were purified using MagneSil™ PMPs and sequenced on an ABI Model 3700 Sequencer. Data are presented as read accuracy for 600 bases for samples from three separate 96 well plates (n = 288). Cultures included DH5α™ cells harboring high-copy number plasmid DNA.

Conclusions

Using the Wizard® MagneSil™ Plasmid Purification System on a robotic platform can provide high quality plasmid DNA in a short time with little to no manual intervention. The procedure as described here involves no manual interventions for processing. The MagnaBot® Magnetic Device, used to manipulate the PMPs during lysate clearing and DNA binding, is affordable and adaptable to most robotic platforms. The automated plasmid DNA purification protocol produces purified plasmid DNA that can be added directly to sequencing reactions with no additional steps, including concentrating the samples. DNA prepared by this method yielded 600 plus-base long reads with accuracy >98%.

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Protocol

- ◆ Wizard® MagneSil™ Plasmid Purification Technical Bulletin #TB286, Promega Corporation. (www.promega.com/tbs/tb286/tb286.html)



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Ordering Information

| Product | Size | Cat.# |
|---|----------|-------|
| Wizard® MagneSil™ Plasmid Purification System | 4 x 96 | A1630 |
| | 8 x 96 | A1631 |
| Wizard® MagneSil™ Plasmid Purification System, HTP1 | 100 x 96 | A1635 |
| MagnaBot® 96 Magnetic Separation Device | 1 unit | V8151 |

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(a)U.S. Pat. No. 6,027,945 and other patents pending

Technical Questions?

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