Be A "Wizard" At Transfection

Wizard MagneSil Tfx™ System for the Purification of Transfection-Grade DNA

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Abstract

The Wizard MagneSil Tfx™ System provides a reliable method for the rapid isolation of plasmid DNA in a multiwell format. The purified plasmid can be used directly for transfection, as well as for other standard molecular biology techniques. A novel endotoxin removal resin ensures high-quality plasmid DNA suitable for transfecting eukaryotic cells. The use of MagneSil™ Paramagnetic Particles for lysate clearing as well as DNA capture circumvents the need for centrifugation or vacuum manifolds, allowing DNA purification with the Wizard MagneSil Tfx™ System to be completely automated.

The Wizard MagneSil Tfx™ System incorporates MagneSil™ Paramagnetic Particle technology to purify high-quality plasmid DNA suitable for transfection of eukaryotic cells.

Introduction

High-throughput plasmid purification requires rapid, reliable, low-cost systems that provide plasmid of high purity for downstream applications. Silica-based systems are routinely used for plasmid purification due to their speed and ease of use. A number of silica-based systems exist (e.g., the Qiagen Turbo System), including silica-impregnated membranes in vacuum and paramagnetic particle-based systems.

While effective for sequencing, DNA purified with these systems contains contaminants that can have a deleterious effect on downstream applications, such as transfection. One of the main contaminants is lipopolysaccharide endotoxin, which has been shown to decrease transfection efficiency in many cell lines.

Anion exchange methods (e.g., the Qiagen Ultra System) isolate high-quality transfection-grade DNA, but are time-consuming and expensive.

We have developed a new plasmid purification system, the Wizard MagneSil Tfx™ System (Cat.# A2380, A2381), that incorporates MagneSil™ Paramagnetic Particle[a] technology to purify high-quality plasmid DNA suitable for transfection of eukaryotic cells.

MagneSil™ Paramagnetic Particles are used to clear bacterial lysates and bind plasmid DNA. In addition, the Wizard MagneSil Tfx™ System employs a novel endotoxin removal resin to lower endotoxin levels, producing highly purified plasmid DNA.

Transfection with plasmid DNA purified using the Wizard MagneSil Tfx™ System results in transfection efficiencies indistinguishable from those of anion exchange-isolated plasmid DNA for a variety of mammalian cell types. This high-quality DNA is suitable for other molecular biology applications including sequencing and restriction enzyme digests.

The Wizard MagneSil Tfx™ System has been designed for use on an automated platform (e.g., Beckman Biomek® FX) in 96-well plate format. No manual intervention is needed once pelleted cells are placed on the robot.

In this article we show yield, endotoxin contaminant and transfection study results obtained using the Wizard MagneSil Tfx™ procedure and compare these results to data generated with plasmid purification systems from Qiagen. For the purpose of this article, Qiagen systems were performed manually following the manufacturer's instructions, while the Wizard MagneSil Tfx™ System was used on automated Beckman workstations.

Preparing Bacterial Cell Cultures

A number of factors including plasmid copy number and cell density affect the final yield of plasmid DNA. For high-throughput applications, bacterial cells are typically grown in 96-well deep-well plates. When grown in 0.25 to 1.0ml of Terrific Broth overnight with agitation, cultures yield adequate biomass for processing. (Sufficient biomass can also be achieved by shaking the cultures.) Because the Wizard MagneSil Tfx™ System uses Paramagnetic Particles to clear lysate, it is important to not overgrow the cells; too much biomass (≥ 8 O.D.$_{600}$) can result in poor lysate clearing.

We used host bacterial strain JM109 containing the high-copy number pGL3 Control Vector[b,c] plasmid (Cat.# E1741). (Optimum parameters for each bacterial cell-plasmid combination will need to be determined.) After cultures were grown, cells were pelleted by centrifugation and the media was decanted.

Protocol Overview

The Wizard MagneSil Tfx™ System protocol includes two steps designed to decrease contaminant levels and increase transfection efficiency. A summary of the protocol is shown below. MagneSil™ BLUE[a] is used to clear lysate and MagneSil™ RED[a,d] is used to bind plasmid. In addition, lysate is treated with Endotoxin Removal Resin (Cat.# A2191), and 4/40 Wash Solution (Cat.# A2221) containing isopropanol and guanidine serves to eliminate other contaminants. Through the use of these reagents, the resulting plasmid is of very high quality.

Wizard MagneSil Tfx™ Protocol

Pellet cells (2–6 O.D.$_{600}$)
↓
Resuspend, lyse, neutralize
↓
Clear bacterial lysate with MagneSil™ BLUE
↓
Endotoxin Removal Resin
↓
Bind DNA with MagneSil™ RED
↓
4/40 Wash
↓
3X ethanol wash
↓
Dry
↓
Elute

High Plasmid DNA Yields

An important factor when choosing a plasmid purification system is how consistently the system purifies high yields of DNA from multiple samples. To check for consistent DNA yields, the Wizard MagneSil Tfx™ System was tested by three users. For these studies we used 96-well plates with 4 O.D.$_{600}$ per well of JM109 cells containing pGL3 Control Vector DNA. The plates consistently yielded in excess of 6µg of DNA (Figure 1).

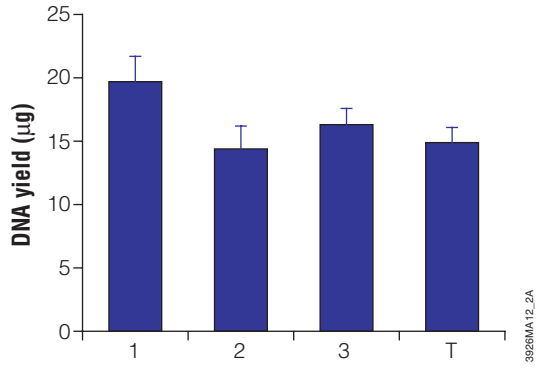


Figure 1. Consistent plasmid yield. Deep-well plates containing 4 O.D.$_{600}$ of JM109 cells transformed with pGL3 Control Vector DNA were processed by three different users (1, 2 and 3) following the standard Wizard MagneSil Tfx™ protocol. For comparison purposes, an identical plate was processed using the Qiagen Turbo system (T). Each bar is the average of 8 samples. Samples were quantitated by gel electrophoresis.

Effective Removal of Endotoxin, RNA and Protein

A number of contaminants including endotoxin, RNA and protein can result in purified plasmid DNA with reduced transfection efficiency. Thus it is critically important to isolate plasmid DNA containing as little of these contaminating agents as possible.

To demonstrate low contaminant levels, identical plates containing JM109 cells with pGL3 Control Vector plasmid were processed using the Wizard MagneSil Tfx™ System and the Qiagen Turbo and Ultra systems. RNA contamination was determined using an HPLC method, protein contamination by a standard Bradford microplate assay (Pierce Cat.# 23236) and endotoxin contamination by a colorimetric assay (Pyrochrome®; Associates of Cape Cod, Cat.# C0180). Levels of RNA and protein in the Wizard MagneSil Tfx™ System preps were similar to the levels present in Qiagen Turbo and Ultra preps (data not shown). Endotoxin results are shown in Figure 2. The data show that Wizard MagneSil Tfx™ System's contaminant-removing steps markedly reduce the amount of endotoxin contaminant in purified plasmid preps.

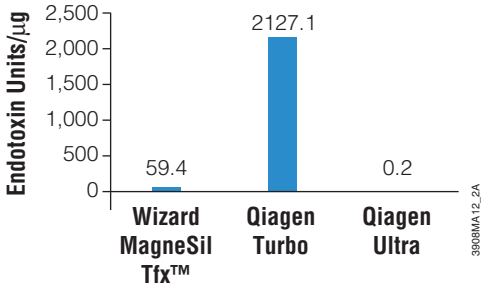


Figure 2. Comparison of endotoxin levels in DNA purified using three plasmid purification systems. Four 96-well plates with 4 O.D.$_{600}$ per well of JM109 cells containing pGL3 Control Vector plasmid DNA were processed using the Wizard MagneSil Tfx™ System and the Qiagen Turbo and Ultra systems. Endotoxin was measured using a colorimetric Pyrochrome® assay (Associates of Cape Cod; Cat.# C0180). Results are shown as endotoxin units per microgram of DNA yield.

Transfection-Grade DNA

For purposes of comparison, we transfected NIH3T3 cells (Figure 3, Panel A), as well as a number of other cell lines (Figure 3, Panel B), using plasmid purification with either a silica membrane-based system (Qiagen Turbo), a system employing anion exchange chemistry (Qiagen Ultra) or the Wizard MagneSil Tfx™ System. Plasmid DNA purified using the Wizard MagneSil Tfx™ System transfected a range of mammalian cell lines as well as or better than anion exchange methods in five commonly used cell lines.

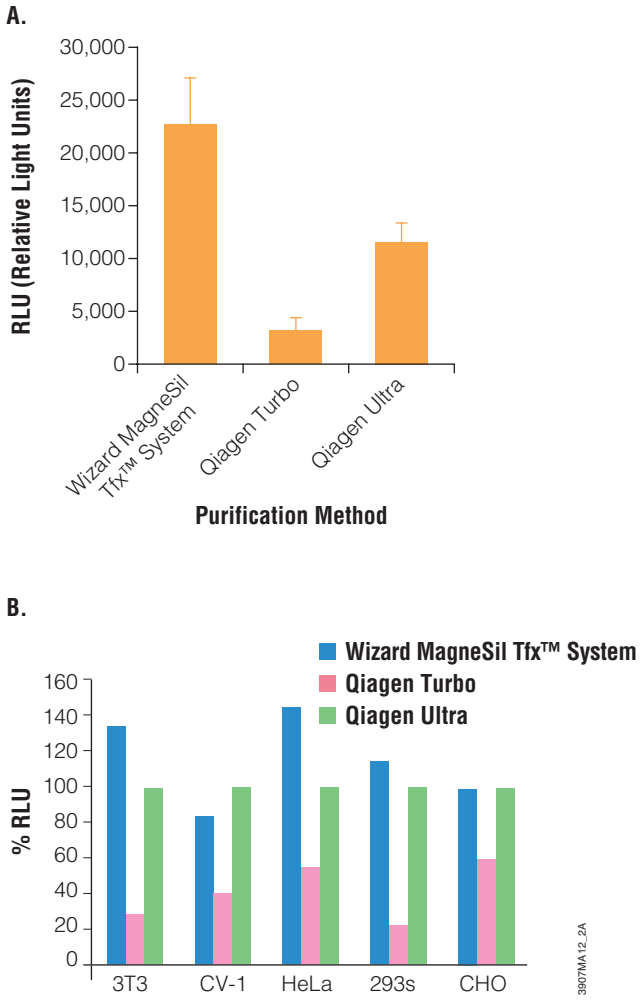


Figure 3. Comparison of transfection results using DNA purified with three plasmid DNA purification systems. Panel A. Transfection of NIH3T3 cells. pGL3 Control Vector DNA was purified by Wizard MagneSil Tfx™, Qiagen Turbo or Qiagen Ultra systems and compared for its efficiency in transformation of NIH3T3 cells using TransFast™ Transfection Reagent[e] (Cat.# E2431). Cells were lysed with Cell Culture Lysis 5X Reagent (Cat.# E1531) after 2 days of growth and assayed using Luciferase Assay System[f,g] 10-pack (Cat.# E1501). Light output, measured in relative light units (RLU), was read using a Labsystems Luminoskan® luminometer.

Panel B. Transfection of a variety of cell lines. A variety of eukaryotic cell lines were transfected with pGL3 Control Vector plasmid purified using the magnetic particle-based Wizard MagneSil Tfx™ System, the silica membrane-based Qiagen Turbo system or the anion exchanged-based Qiagen Ultra system. Transfection efficiency was normalized as a percentage of anion exchange and is shown as a function of light output.

Conclusions

Using the Wizard MagneSil Tfx™ System on a robotic platform can provide high-quality plasmid DNA rapidly and efficiently. The purified DNA has low amounts of common contaminants and is very efficient in transfection experiments.

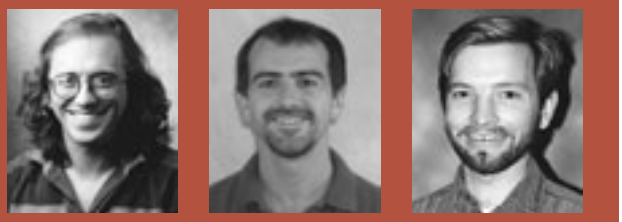
Protocols have been developed on the Beckman Biomek® FX workstation and Beckman Biomek® 2000, resulting in purified plasmid DNA of equal purity and transfection efficiency to DNA purified manually. In addition, adaptation to other robotic workstations is feasible.

Comparison of the Wizard MagneSil Tfx™ System with other commercially available systems shows that the Wizard MagneSil Tfx™ System provides high-quality plasmid DNA similar to plasmid DNA purified using anion exchange methods. The Wizard MagneSil Tfx™ System provides an attractive alternative to filtration-based plasmid purification systems.

For the robotic method, go to: **www.promega.com/automethods** and provide your contact information. An automated Support Team member will then contact you regarding a robotic method for your system.

Protocol

- *Wizard MagneSil Tfx™ System Technical Bulletin*, #TB314, Promega Corporation. (www.promega.com/tbs/tb314/tb314.html)



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Ordering Information

Product	Size	Cat.#
Wizard MagneSil Tfx™ System	4 × 96 preps	A2380
	8 × 96 preps	A2381

[a] U.S. Pat. Nos. 6,027,945 and 6,368,800, Australian Pat. No. 732756 and other patents and patents pending.

[b] U.S. Pat. No. 5,670,356.

[c] The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673.

[d] U.S. Pat. No. 6,284,470 and other patents pending.

[e] The cationic lipid component of the TransFast™ Transfection Reagent is covered by U.S. Pat. Nos. 5,824,812, 5,869,715 and 5,925,623, Australian Pat. No. 713093 and pending foreign patents.

[f] U.S. Pat. Nos. 5,283,179, 5,641,641, 5,650,289 and 5,814,471, Australian Pat. No. 649289 and other patents and patents pending.

[g] Certain applications of this product may require licenses from others.