Pure Yield Meets Pure Speed

Promega

Fast, Reliable, High-Quality Midiprep Plasmid Purification Using the PureYield™ Plasmid Midiprep System

By Doug White, M.S., Cheryl Bailey, Ph.D., Hemanth Shenoi, Ph.D., and Rex Bitner, Ph.D., Promega Corporation

Abstract

This article introduces the PureYield™ Plasmid Midiprep System. The system is designed to rapidly isolate highly pure plasmid DNA for transfection, in vitro expression, automated fluorescent DNA sequencing and other applications. The unique design of the new PureYield™ columns yields 100–200µg of plasmid DNA in less than 30 minutes and elutes in a concentrated volume, eliminating the need to ethanol precipitate plasmid DNA prior to use. An Alternative Lysate Clearing Protocol can extend the system to purify up to 400µg of plasmid DNA. The PureYield™ System can save up to three-and-a-half hours compared to other plasmid midiprep methods freeing, researchers to focus on more challenging aspects of their work.

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Introduction

As research moves from DNA sequencing to expression analysis, the need has increased for rapid methods to isolate hundreds of micrograms of high-quality plasmid DNA for use in eukaryotic transfection and in vitro expression experiments. The PureYield™ Plasmid Midiprep System[a] rapidly purifies midiprep amounts of plasmid DNA allowing you to spend time on the more challenging aspects of your work.

The system provides a fast, simple technique using a newly developed silica membrane column for purification of 100–200µg of pure plasmid DNA from *E. coli* cultures. Plasmid DNA can be purified in less than 30 minutes, greatly reducing the time spent on purification compared to resin or other membrane column methods.

The PureYield™ Plasmid Midiprep System also incorporates a unique Endotoxin Removal Wash solution step designed to remove substantial amounts of endotoxin, protein and RNA contaminants that may interfere with highly sensitive applications such as transfection and TNT® coupled in vitro transcription/translation.[b–f] Purification is achieved without isopropanol precipitation of purified plasmid DNA or extensive high-speed centrifugation, providing concentrated, pure plasmid DNA in one rapid and easy method.

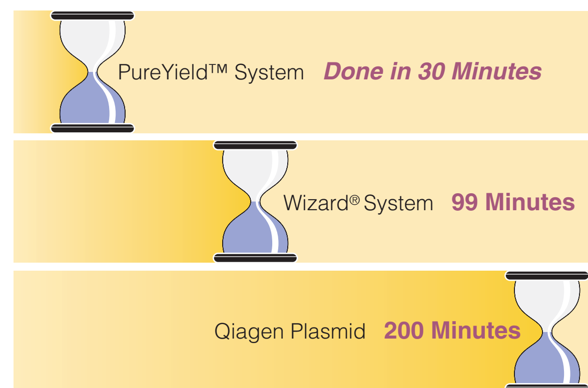


Figure 1. Comparison of time required per midiprep using different systems. Each system protocol was performed according to the manufacturer's instructions using 50ml of an overnight culture of JM109 bacteria transformed with a high-copy plasmid (pGEM®-3)[g]. For the PureYield™ System, the vacuum protocol was used. Total time to perform the midiprep is noted.

Procedure

The PureYield™ Plasmid Midiprep System provides pure plasmid DNA in significantly less time than other systems (Figure 1). The method was designed to minimize both hands-on and total time. For example, the PureYield™ Lysate Clearing and DNA Binding Columns have high flow rates, eliminating the need for high-speed centrifugation steps.

The PureYield™ System purifies plasmid DNA from *E. coli* cultures using either a low-speed, swinging-bucket centrifugation method, a vacuum method, or a combination of these two methods to provide maximum flexibility. You can tailor the system to your needs based on available equipment, balance of time vs. yield, and the culture volume being processed. The vacuum method is faster than the centrifugation method, but the centrifugation method clears lysates from samples with a large biomass (culture volume or cell number) more efficiently. The combination protocol has the speed of the vacuum protocol for the binding step but uses a low-speed centrifugation step for more efficient lysate clearing. All three methods provide high-quality plasmid DNA and give similar yields for low biomass samples (Figure 2).

To isolate plasmid DNA from a 50ml culture, collect cells by centrifugation, resuspend the cells in buffer and lyse under gentle alkaline conditions. Lysate clearing is accomplished using the PureYield™ Clearing Column with either the low-speed swinging-bucket centrifugation method or the vacuum method where a "stacked column" approach couples lysate clearing and plasmid DNA

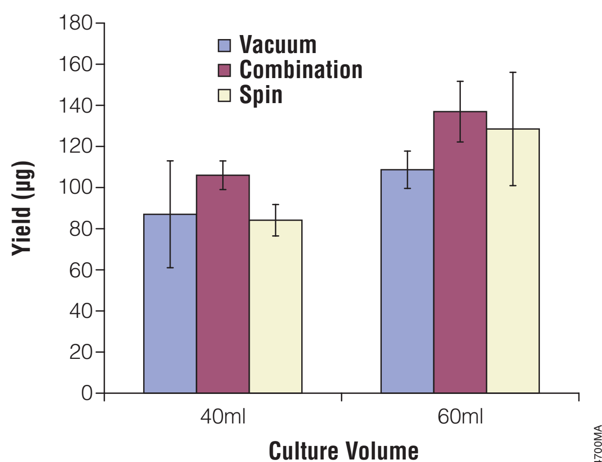


Figure 2. Comparison of yields using the three PureYield™ protocols. The indicated amount of JM109 culture containing a pGEM® Vector was processed using each of the three PureYield™ protocols. Yields calculated using the absorbance at 260nm.

binding to the silica membrane in the PureYield™ Binding Column in one step (Figure 3). This process occurs sequentially when using the centrifugation method. After plasmid DNA binds to the silica membrane, the membrane is washed first with Endotoxin Removal Wash to remove endotoxin, RNA and protein contaminants followed by the Column Wash to remove additional impurities. Concentrated, pure plasmid DNA is eluted directly from the silica membrane in room-temperature Nuclease-Free Water and is ready for eukaryotic transfection, in vitro coupled transcription/translation, fluorescent DNA sequencing or other applications without requiring isopropanol precipitation or other manipulation.

Figure 3. Proper assembly of the PureYield™ Clearing Column (blue) and the PureYield™ Binding Column (white) for use with the PureYield™ Plasmid Midiprep System vacuum protocol.

Yield and Purity

Plasmid DNA purified from transformed *E. coli* cultures was analyzed for yield and purity by measuring sample absorbance at 260nm and 280nm. Plasmid DNA yield was calculated using the absorbance at 260nm. Purity was estimated from the ratio of absorbance at 260nm and 280nm (A_{260}/A_{280} ratio) and agarose gel analysis. Pure plasmid DNA exhibits an A_{260}/A_{280} ratio of 1.8–1.9. Table 1 shows the average yield and purity of plasmid DNA purified from 50ml of an overnight culture of JM109 cells using the PureYield™ Plasmid Midiprep System.

Table 1. Yield and purity of plasmid DNA purified with the PureYield™ Plasmid Midiprep System.

Sample	A_{260}/A_{230}	A_{260}/A_{280}	µg/ml	Yield (µg)
1	1.93	1.86	251	128
2	1.79	1.73	270	143
3	2.02	1.89	293	147
4	2.15	1.90	266	141
5	1.81	1.72	203	142
6	2.12	1.89	257	141
average	1.97	1.83	257	140
Std. Dev.	0.15	0.08	30	6
%CV	8	5	12	5

Absorbance at 260nm is not always an accurate measure of plasmid yield. Contaminants, such as RNA and protein, can also absorb at 260nm, creating an overestimation of yield. This is especially true when the plasmid concentration is low compared to potential contaminants (e.g., purifying low-copy-number plasmids). We compared yields estimated by A_{260} and agarose gel analysis for the PureYield™ System and other plasmid prep methods (data not shown). Certain methods had a yield determined by A_{260} that was twice the yield found by gel analysis, showing that A_{260} is not always an accurate method for determining yield. In contrast, the A_{260} to gel analysis yield ratio using the PureYield™ System was 1–1.2, giving users confidence that A_{260} yield determinations are accurate.

Purity, as measured by the average A_{260}/A_{280}, using the PureYield™ System was between 1.8 and 1.9 (Table 1). No degradation was observed when purified plasmid DNA was incubated at 37°C for 16 hours with MULTI-CORE™ Buffer and then visualized by agarose gel electrophoresis, indicating no detectable carryover of endonucleases. Agarose gel analysis of purified high-copy-number plasmid also shows greater than 90% supercoiled plasmid (data not shown).

To further test the system, we compared plasmid DNA yields from increasing amounts of bacterial culture volume to determine the binding capacity of the system. Using the Alternative Lysate Clearing Protocol, which incorporates a high-speed centrifugation for lysate clearing, yields doubled to 400µg using 180ml or 240ml of bacterial culture (Figure 4). This protocol allows the PureYield™ System to purify far more plasmid than other midiprep methods, which have a typical binding capacity of only 100–200µg.

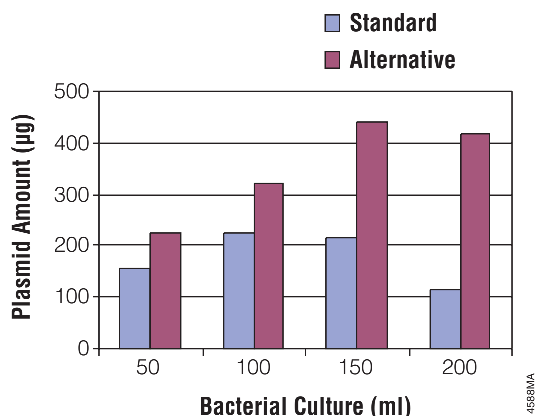


Figure 4. Yield of pGEM® plasmid purified from increasing amounts of JM109 bacterial culture using two different lysate preparation methods. JM109 cells containing pGEM® plasmid were grown in LB media overnight. Lysates were created as described in Technical Manual #TM253 by the standard or alternative protocols and cleared using vacuum purification. As biomass (culture volume and cell number) increases, we recommend the alternative clearing method over the standard lysate protocol.

Transfection of Eukaryotic Cells

Perhaps the most common application of midiprep plasmid DNA is transfection of eukaryotic cells. Successful transfection relies on highly purified plasmid DNA containing low levels of endotoxin. In this experiment, the psiCHECK™-2 Vector (Cat.# C8021) carrying a Firefly luciferase gene was purified using either the PureYield™ System, the HiSpeed™ Qiagen Plasmid Midi Kit or the Eppendorf Perfectprep® Plasmid Midi Kit and transfected into HeLa cells. Plasmid DNA isolated with the PureYield™ System demonstrated higher gene expression levels of Firefly luciferase, as measured by luminescence in the transfected HeLa cells.

In Vitro Protein Expression Using Plasmid DNA Purified with the PureYield™ System in a TNT® System Reaction

A common method to confirm open reading frames determined by DNA sequencing is to express the proteins in vitro. One of the most robust and simple methods to perform in vitro protein expression is to use the TNT® Quick Coupled Transcription/Translation Systems[b–f]. Plasmid DNA was purified from JM109

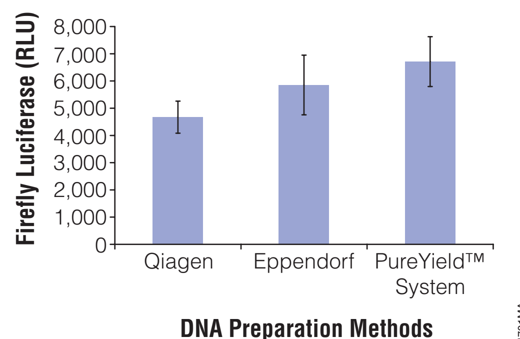
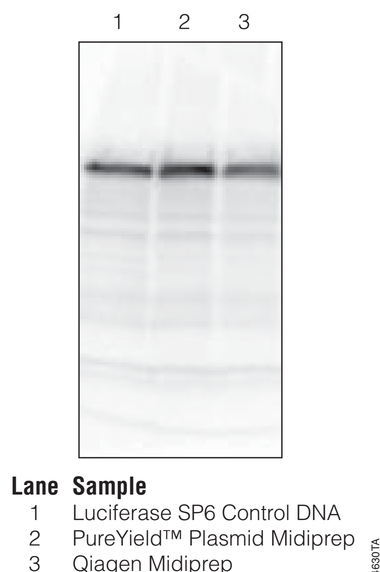


Figure 5. Comparison of transfection of plasmid DNA purified with the PureYield™ System and other midiprep systems. psiCHECK™-2 Vector (Cat.# C8021), which carries a Firefly luciferase gene, was isolated from *E.coli* using either the PureYield™ System, the HiSpeed™ Qiagen Plasmid Midi Kit or the Eppendorf Perfectprep® Plasmid Midi Kit. HeLa cells were transfected using 0.07µg of DNA in a total of 25µl. The Firefly luciferase signal was monitored with the Dual-Glo™ Luciferase Reporter System (Cat.# E1910).

bacteria transformed with SP6-control plasmid using the PureYield™ System and compared to plasmid DNA isolated using other systems. Protein expression was performed as described in the *TNT® Quick Coupled Transcription/Translation System Technical Manual* #TM045. Figure 6 shows robust protein expression compared to control plasmid provided in the TNT® System as well as to expression using plasmid DNA purified by other methods.

Applications of Plasmid DNA Purified Using the



Lane	Sample
1	Luciferase SP6 Control DNA
2	PureYield™ Plasmid Midiprep
3	Qiagen Midiprep

4630TA

Figure 6. Comparison of in vitro transcription/translation results of purified plasmid DNA prepared using different midiprep systems. Midipreps of the Luciferase SP6 Control DNA (Cat.# L4741) were performed as described by the manufacturer. Transcription/translation reactions were performed as described in the *TNT® Quick Coupled Transcription/Translation System Technical Manual* #TM045. One microliter of each TNT® reaction was analyzed by SDS-PAGE on a 4–20% NOVEX® gel. The separated proteins were transferred to a PVDF membrane (BioRad Sequi-Blot™) and then exposed for two hours to a PhosphorImager® cassette. A Storm® PhosphorImager® was used to analyze the cassette.

PureYield™ Plasmid DNA Midiprep System: Fluorescent Automated DNA Sequencing

Automated fluorescent DNA sequencing requires high-quality plasmid DNA at an appropriate concentration. To assess plasmid purified with the PureYield™ System in this application, prepared samples were sent to SeqWright (Houston, TX) for automated fluorescent DNA sequencing. Sequencing was performed on an ABI 3730 fluorescent sequencer using ABI BigDye® chemistry. Purified plasmid DNA produced a phred quality score of 20–30 at 700 bases, indicating a 99% accuracy of sequence results (data not shown).

Conclusion

The PureYield™ Plasmid Midiprep System is a fast and simple system for medium-scale plasmid DNA purification. The system uses a new silica membrane design that takes less time and produces higher yields than comparable midipreps. The eluted DNA can be used directly in applications without the need for alcohol precipitation. The system provides consistent quality for demanding applications such as eukaryotic transfection, in vitro expression and fluorescent DNA sequencing.

Protocols

- *PureYield™ Plasmid Midiprep System Technical Manual* #TM253, Promega Corporation. (www.promega.com/tbs/tm253/tm253.html)
- *TNT® Quick Coupled Transcription/Translation Systems Technical Manual* #TM045, Promega Corporation. (www.promega.com/tbs/tm045/tm045.html)

Ordering Information

Product	Size	Cat.#
PureYield™ Plasmid Midiprep System[(a)]	25 preps	A2492
	100 preps	A2495
Vac-Man® Jr. Laboratory Vacuum Manifold, 2-sample capacity	1 each	A7660
Vac-Man® Laboratory Vacuum Manifold, 20-sample capacity	1 each	A7231

(a) U.S. Pat. No. 6,194,562, Australian Pat. No. 740145, Canadian Pat. No. 2,329,067 and other patents pending.

(b) U.S. Pat. Nos. 5,324,637 and 5,492,817, Australian Pat. No. 660329 and other patents.

(c) U.S. Pat. Nos. 5,283,179, 5,641,641, 5,650,289 and 5,814,471, Australian Pat. No. 649289 and other patents.

(d) U.S. Pat. Nos. 4,966,964, 5,019,556 and 5,266,687, Australian Pat. Nos. 616881 and 641261 and other pending and issued patents, which claim vectors encoding a portion of human placental ribonuclease inhibitor, are exclusively licensed to Promega Corporation.

(e) 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. A license (from Promega for research reagent products and from The Regents of the University of California for all other fields) is needed for any commercial sale of nucleic acid contained within or derived from this product.

(f) U.S. Pat. No. 5,552,302, Australian Pat. No. 646803 and other patents.

(g) U.S. Pat. No. 4,766,072.

(h) Licensed from University of Georgia Research Foundation, Inc., under U.S. Pat. Nos. 5,292,658, 5,418,155, Canadian Pat. No. 2,105,984 and related patents.

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Doug White, M.S.
Research Scientist

Hemanth Shenoi, Ph.D.
Product Manager

Rex Bitner, Ph.D.
Technology Manager

Cheryl Bailey, Ph.D.
Research Scientist