TRANSFECTION-QUALITY PLASMID DNA IN AS LITTLE AS TEN MINUTES USING THE PUREYIELD™ PLASMID MINIPREP SYSTEM

DON SMITH AND ERIC VINCENT, PROMEGA CORPORATION

Here we introduce the PureYield™ Plasmid Miniprep System that allows researchers to isolate high-quality plasmid DNA suitable for a broad range of downstream applications, including transfection, in vitro expression, sequencing and cloning. The system allows direct purification of plasmid from as much as 0.6 ml of bacterial culture without cell harvesting or from up to 3 ml of culture when the cells are pelleted. The entire procedure can be completed in as little as 10 minutes.

Introduction

Miniprep plasmid purification is a primary research tool both for gene manipulation and protein expression. However, the purity and concentration obtained with most commercially available miniprep systems limits the use of the plasmid to less demanding applications. The PureYield™ Plasmid Miniprep System produces high-quality DNA useful for even the most demanding applications, including transfection. The superior quality of the isolated plasmid DNA allows researchers to avoid large-scale preps.

The unique reagents, proprietary matrix and column design of the PureYield™ Plasmid Miniprep System allow rapid purification of DNA directly from bacterial culture in less than 10 minutes with elution volume as low as 30 µl, resulting in a more concentrated plasmid stock. The low elution volume is possible because the column design retains virtually no volume. Eluted plasmid is no longer contaminated with leftover wash solution, salts or alcohols, allowing use of the purified plasmid for highly sensitive applications such as transfection and TnT® coupled in vitro transcription/translation. An additional benefit is that the same degree of purification can be obtained even with low-copy plasmids. Although the system works best for plasmids less than 10 kb, plasmids as large as 18 kb have been purified.

Obtain High-Quality DNA in 10 Minutes

The unique combination of reagents in the PureYield™ Plasmid Miniprep System allows purification of plasmid either directly from bacterial culture or from cell pellets representing as much as 3 ml of cell culture (Figure 1). A typical overnight culture is grown in LB for 16–18 hours, and a total of 0.6 ml is used for the direct isolation method. If a larger volume is chosen, cells are first harvested by centrifugation, then resuspended in 600 µl of medium or water. A 100 µl volume of Lysis Buffer is added and mixed by inversion until a clear blue solution is obtained. Next, 350 µl of chilled Neutralization Solution is added, and the solution is mixed by inverting the tube until a uniform yellow color is obtained, indicating a complete pH change. A heavy precipitate will form as a result of the neutralization and is cleared by centrifugation in a conventional microcentrifuge. Cleared lysates are transferred into purification columns set in collection tubes. These samples are centrifuged for 20 seconds. The columns are removed temporarily, and the column flowthroughs are discarded. The columns are replaced into the collection tubes. Twenty microliters of Endotoxin Removal Wash is added to each column followed by a brief 15-second centrifugation. Without removing the columns from the centrifuge, 400 µl of Column Wash Buffer is added, and centrifugation is repeated for 30 seconds. The columns are transferred to clean 1.5 ml tubes, and 30 µl of Elution Buffer is applied. Following a one-minute incubation, plasmid DNA is collected by centrifugation.

Miniprep Plasmid System Yields High-Purity DNA

We purified the high-copy pGEM®-3Zf(+) plasmid from *E. coli* cells using the PureYield™ Plasmid Miniprep System. The purified DNA was analyzed spectrophotometrically, estimating plasmid yields by absorbance at 260 nm. Purity of the plasmid was judged by both the $A_{260/280}$ ratio, typically 1.8–1.9 for pure plasmid, and by the $A_{260/230}$ ratio, typically greater than 2.0 for pure plasmids. Lower values are indicative of protein contamination ($A_{260/280}$) or of salt and/or ethanol contamination ($A_{260/230}$). Table 1 presents the yield and purity of the plasmid DNA obtained. Additionally, agarose gel analysis was used to look at the integrity of the purified plasmid (Figure 2, Panel A). To determine whether yield can be improved further by increasing elution volume, we performed plasmid isolations from 0.6 ml cultures of JM109 cells carrying the pGEM®-3Zf(+) plasmid. A slight yield advantage was seen by increasing elution volume to 100 µl (Figure 2, Panel B), but no additional advantage was gained from elution volumes greater than 100 µl (data not shown).

Miniprep Plasmid DNA Gives Superior Performance in Coupled Transcription/Translation Systems

We tested the performance of plasmid DNA isolated using the PureYield™ Plasmid Miniprep System in the TnT® T7 Quick Coupled Transcription/Translation System (Figure 3). Luciferase T7 Control Plasmid was purified from 1.0 or 1.5 ml of JM109 cells. The DNA obtained from the PureYield™ Miniprep System was compared to control plasmid supplied with the transcription/translation system and plasmid purified using a spin/vacuum system. The PureYield™ Miniprep plasmid DNA performed as well as the control DNA supplied with the transcription/translation system. Plasmid DNA

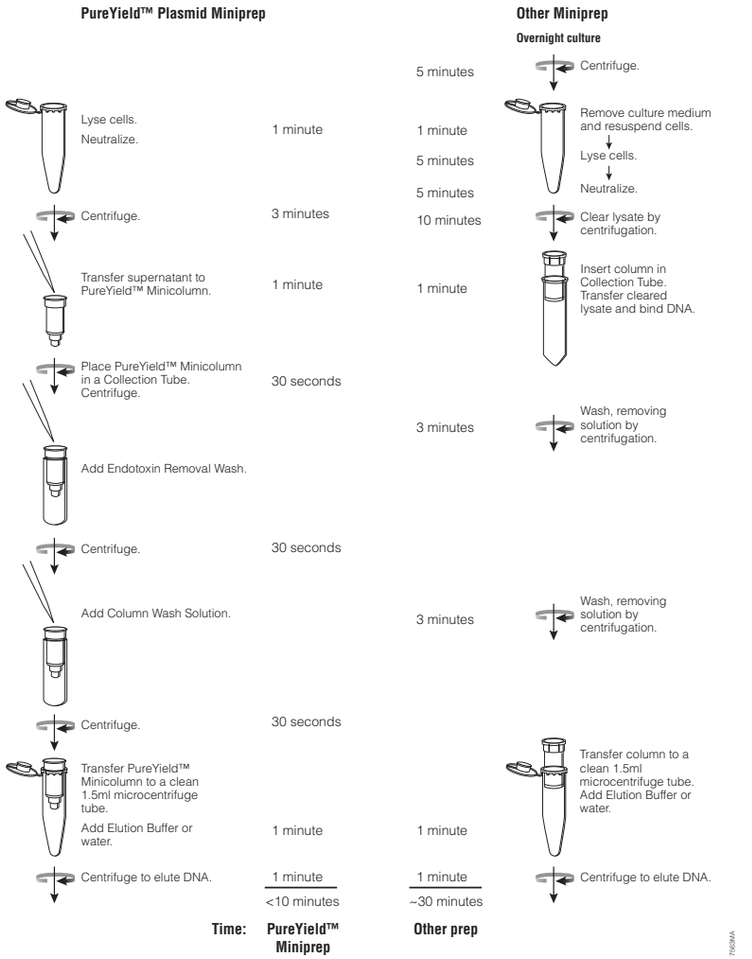

Figure 1. The PureYield™ Plasmid Miniprep System yields transfection-quality DNA in approximately 10 minutes.

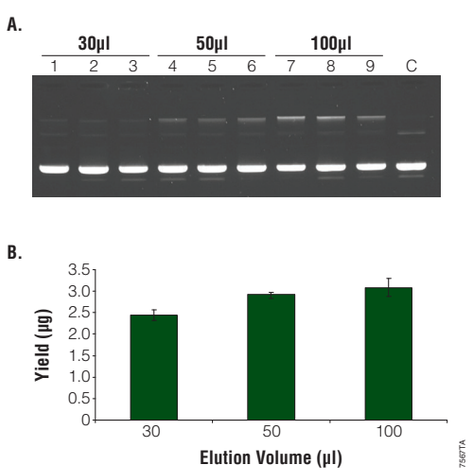

Figure 2. Integrity of plasmid obtained using the PureYield™ Plasmid Miniprep System. Plasmid was purified from 0.6 ml of JM109 culture, and 1 µg was loaded onto an agarose gel and visualized by ethidium-bromide staining (**Panel A**). Lanes 1–3, 30 µl elution volume; 4–6, 50 µl elution volume; 7–9, 100µl elution volume. Lane C, control plasmid from a large-scale industrial prep. Yield can be increased slightly by elution volumes up to 100 µl (**Panel B**). N = 6 at each elution volume.

Table 1. Purity of Plasmid Isolated Using the PureYield™ Plasmid Miniprep System.

Culture Volume	Attribute	$A_{260/230}$	$A_{260/280}$	Yield (µg)
0.6 ml	Mean	2.16	1.85	2.44
	Standard Deviation	0.179	0.023	0.315
	CV	8.27%	1.26%	12.9%
1.0 ml	Mean	2.31	1.87	5.43
	Standard Deviation	0.019	0.012	0.303
	CV	0.80%	1.26%	5.58%
1.5 ml	Mean	2.29	1.86	7.78
	Standard Deviation	0.022	0.004	0.741
	CV	0.95%	0.22%	9.53%

N = 6 preps for each volume of starting material.

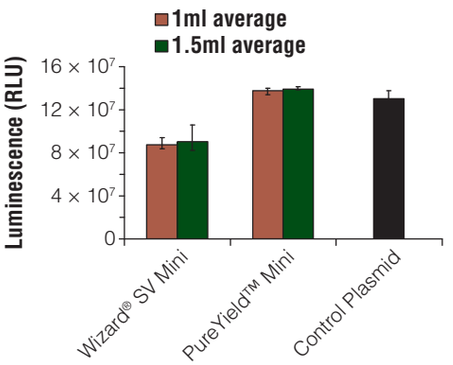

Figure 3. Plasmid DNA prepared using the PureYield™ Plasmid Miniprep System works as well as control plasmid DNA in coupled transcription/translation reactions. Luciferase T7 Control Plasmid DNA was purified from 1.0 ml or 1.5 ml of JM109 cells using either the Wizard® *Plus* SV Miniprep DNA Purification System or the PureYield™ Plasmid Miniprep System, then used in the TNT® T7 Quick Coupled Transcription/Translation System and compared to the control DNA supplied with the TNT® Quick System. Protein expression was quantitated with the ONE-Glo™ Luciferase Assay System. (N = 6).

purified using the PureYield™ Miniprep System also performed well in automated sequencing, giving perfect reads of greater than 700 bases (Figure 4).

One of the greatest assets of the PureYield™ Plasmid Miniprep System is the ability to prepare transfection-grade DNA using a small-scale, rapid method. Figure 5 shows data comparing plasmids prepared using either a spin/vacuum miniprep system or the PureYield™ Plasmid Miniprep System. The purified plasmid was used to transfect several common mammalian cell lines. The most consistent transfection results are from plasmid prepared using the PureYield™ Plasmid Miniprep System. Even problematic cell lines like CHO cells were transfected at good efficiencies.

Summary

The PureYield™ Plasmid Miniprep System gives researchers a new option for preparing small quantities of high-quality DNA suitable for applications such as transfection, cloning, sequencing and coupled transcription/translation. The procedure can be completed in 10 minutes and can be performed directly on 0.6 ml of bacterial cell culture or on harvested cells from up to 3.0 ml of bacterial cell culture.

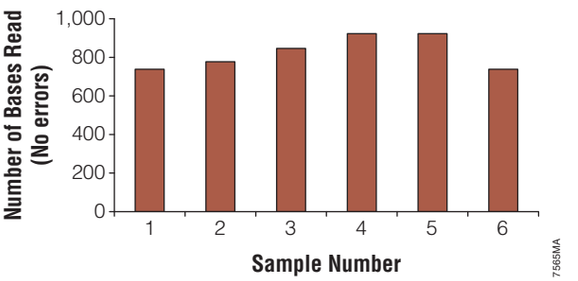

Figure 4. Plasmid DNA isolated using the PureYield™ Plasmid Miniprep System performs well in automated DNA sequencing. Six separate preparations of pGEM®-3Zf(+) plasmid were made using the PureYield™ Plasmid Miniprep System and submitted for contract sequencing. All six preparations gave perfect reads of greater than 700 bases.

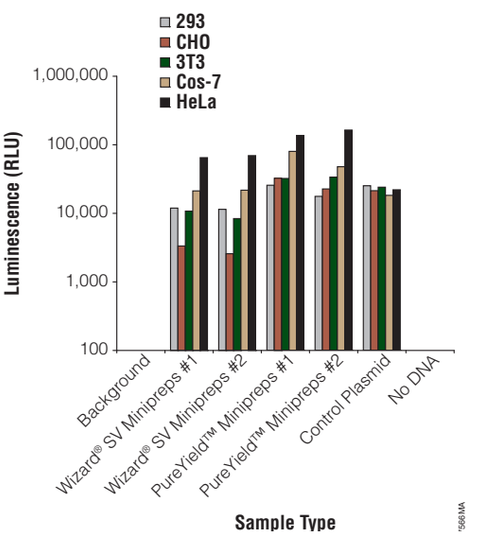

Figure 5. Plasmid DNA prepared using the PureYield™ Plasmid Miniprep System consistently works well in transfection experiments. Promega pGL4.13 plasmid was prepared using a spin/vacuum system or the PureYield™ Plasmid Miniprep System. Five different commonly used mammalian cell lines were transfected with the plasmid, and transfection efficiency was assessed by measuring the luciferase activity of the cell lines using the ONE-Glo™ Luciferase Assay System. (N = 6).

Protocol

PureYield™ Plasmid Miniprep System Technical Bulletin #TB374 (www.promega.com/tbs/tb374/tb374.html)

Ordering Information

Product	Cat.#
PureYield™ Plasmid Miniprep System	Available Soon

Products may be covered by pending or issued patents or may have certain limitations. Please visit our Web site for more information.