Transfection-Quality Plasmid DNA in as Little as Ten Minutes Using the PureYield™ Plasmid Miniprep System

ABSTRACT Scientists require straightforward methods for plasmid purification as the first step in many laboratory protocols. Here we introduce the new PureYield™ Plasmid Miniprep System, which 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.

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INTRODUCTION

Miniprep plasmid purification is a primary research tool for both gene manipulation and protein expression. However, the DNA purity and concentration obtained with most commercially available miniprep systems limit the use of the plasmid to less demanding applications. The PureYield™ Plasmid Miniprep System[a] produces high-quality DNA useful for even the most demanding applications such as transfection.

The unique reagents, proprietary matrix and Minicolumn design of the PureYield™ Plasmid Miniprep System allow rapid DNA purification directly from bacterial culture in less than 10 minutes with elution volumes as low as 30 µl, resulting in more concentrated plasmid DNA. The low elution volume is possible because the column design retains virtually no volume. Leftover wash solution, salts and alcohols are eliminated from the plasmid prep, allowing use of the purified plasmid for highly sensitive applications such as transfection and the S30 T7 High-Yield Protein Expression System. An additional benefit is that the same degree of purification can be obtained even with low-copy-number plasmids. Although the system works best for plasmids less than 10 kb, plasmids as large as 18 kb have been purified.

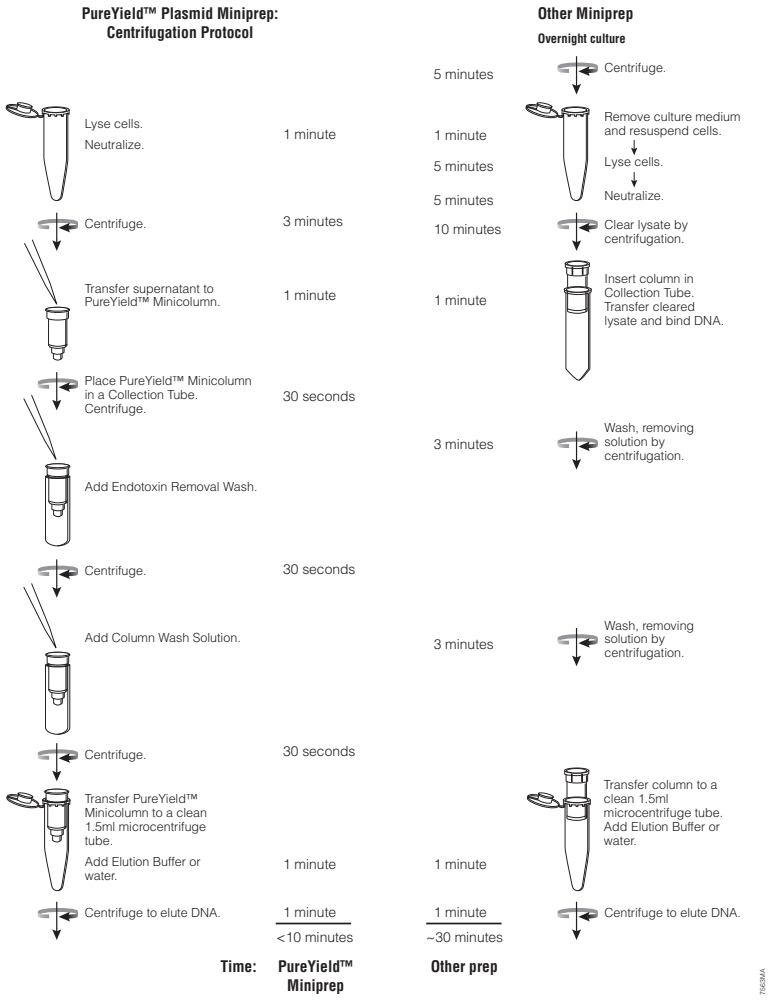

Figure 1. The PureYield™ Plasmid Miniprep System yields transfection-quality DNA in approximately 10 minutes.

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 cell pellets representing as much as 3 ml of cell culture (Figure 1). A typical overnight culture is grown in LB medium for 16–18 hours, and 0.6 ml is used for the direct isolation method. If a larger volume is chosen, cells are harvested by centrifugation, then resuspended in 600 µl of TE buffer or water. Lysis Buffer (100 µl) 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 inversion until a uniform yellow color is obtained, indicating a complete pH change. A heavy precipitate will form as a result of neutralization and is cleared by centrifugation in a conventional microcentrifuge. Cleared lysates are transferred to Minicolumns set in collection tubes, then centrifuged for 20 seconds. The Minicolumns

are removed, and the column flowthroughs are discarded. The Minicolumns are placed back in the collection tubes, then 200 µl of Endotoxin Removal Wash is added to each Minicolumn, followed by a 15-second centrifugation. Without removing the Minicolumns from the centrifuge, 400 µl of Column Wash Buffer is added, and centrifugation is repeated for 30 seconds. The Minicolumns 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 SYSTEM YIELDS HIGH-PURITY DNA

We purified the high-copy pGEM®-3Zf(+) Vector from *E. coli* cells using the PureYield™ Plasmid Miniprep System. Purified DNA was analyzed spectrophotometrically, and yields were estimated by absorbance at 260 nm. Plasmid purity was judged by the $A_{260/280}$ ratio (≥1.8 for pure plasmid), and $A_{260/230}$ ratio (≥2.0 for pure plasmid). Lower values are indicative of protein contamination ($A_{260/280}$) or the presence of guanidine salt or other contaminants ($A_{260/230}$). Table 1 presents the yield and purity of the plasmid DNA obtained. Additionally, agarose gel analysis was used to examine 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(+) Vector. 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). Highest purity plasmid was obtained when bacterial cells were pelleted and resuspended in 600 µl water. Medium components and bacterial growth byproducts can cause lower $A_{260/230}$ ratios and reduce the purity of isolated plasmid DNA.

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.

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

We tested the performance of plasmid DNA isolated using the PureYield™ Plasmid Miniprep System in the S30 T7 High-Yield Protein Expression System (Figure 5). Plasmid DNA (pFN6A-hRL or pFN6A-GFP) was used to transform JM109 bacteria, and plasmid was isolated from 1.5 ml of culture using the PureYield™ Plasmid Miniprep System or a leading competitor system. One

The PureYield™ Plasmid Miniprep System gives you a new option to prepare small quantities of high-quality DNA suitable for transfection, cloning, sequencing or coupled transcription/translation.

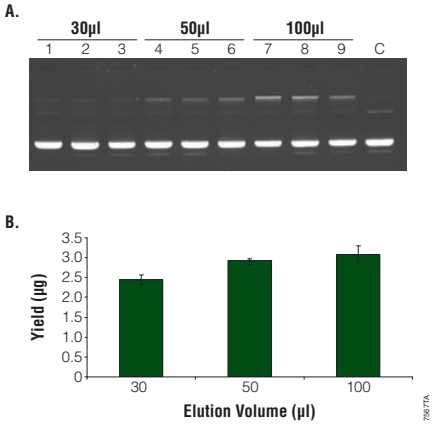

Figure 2. Integrity of plasmid obtained using the PureYield™ Plasmid Miniprep System. Plasmid was purified from 0.6 ml of JM109 culture, then 1 µg was loaded onto an agarose gel and visualized by ethidium-bromide staining (**Panel A**). Lanes 1–3, 30 µl elution volume; 4–6, 50 µl elution volume; 7–9, 100 µl 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.

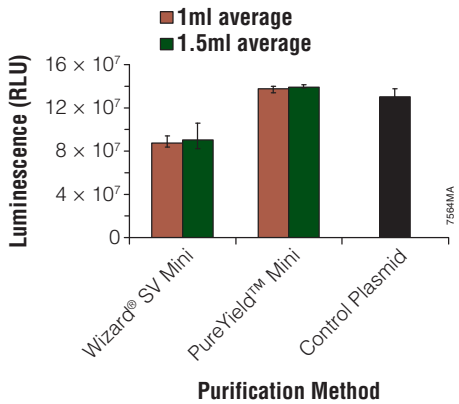

Figure 3. Plasmid DNA prepared using the PureYield™ Plasmid Miniprep System works as well as control plasmid DNA in coupled transcription/translation reactions. Luciferase T7 Control Plasmid DNA was purified from 1.0 ml or 1.5 ml of JM109 cell culture using either the Wizard® *Plus* SV Miniprep DNA Purification System or 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 quantified with the ONE-Glo™ Luciferase Assay System (n = 6).

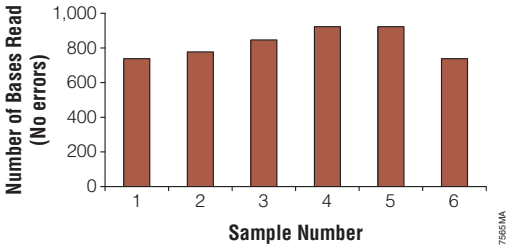

Figure 4. Plasmid DNA isolated using the PureYield™ Plasmid Miniprep System performs well in automated DNA sequencing. Six separate preparations of pGEM®-3Zf(+) Vector were made using the PureYield™ Plasmid Miniprep System and submitted for contract sequencing. All six preparations gave perfect reads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

microgram of plasmid was added to each translation reaction, and protein synthesis was evaluated by SDS polyacrylamide gel electrophoresis (SDS-PAGE) followed by Coomassie® blue staining. The PureYield™ Miniprep plasmid DNA performed similarly to the control DNA provided with the translation system.

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 6 shows data comparing plasmids prepared using a competing system or the PureYield™ Plasmid Miniprep System. The purified plasmid was used to transfect several common mammalian cell lines. The most consistent transfection results were from plasmid prepared using the PureYield™ Plasmid Miniprep System. Even problematic cell lines like CHO cells were transfected with good efficiencies.

The pFN6A-hRL and pFN6A-GFP plasmids encode the 36 kDa *Renilla* luciferase and 26 kDa Monster Green® Fluorescent Protein, respectively.

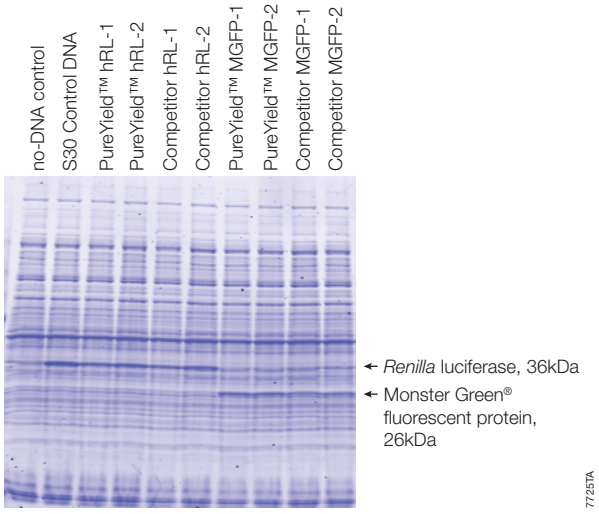

Figure 5. Plasmid DNA prepared using the PureYield™ Plasmid Miniprep System works as well as control plasmid DNA in coupled transcription/translation reactions. pFN6A-hRL or pFN6A-GFP plasmid DNA was isolated from 1.5 ml of JM109 cell culture using the PureYield™ Plasmid Miniprep System or a leading competitor's miniprep. One microgram of plasmid was added to S30 T7 High-Yield Protein Expression System transcription/translation reactions. Protein synthesis of *Renilla* luciferase (36 kDa) and green fluorescent protein (26 kDa) was evaluated by SDS-PAGE followed by Coomassie® blue staining. The PureYield™ Miniprep plasmid DNA performed similarly to the control DNA provided with the translation system.

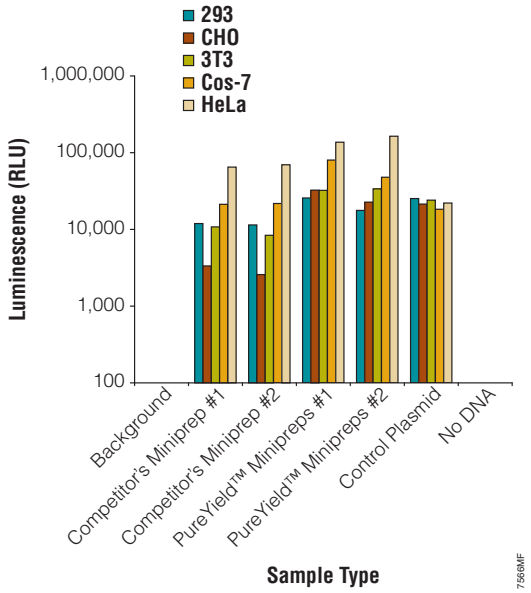

Figure 6. Plasmid DNA prepared using the PureYield™ Plasmid Miniprep System consistently works well in transfection experiments. Promega pGL4.13[*luc2*/SV40] Vector was prepared using a competing system or the PureYield™ Plasmid Miniprep System. Five different commonly used mammalian cell lines were transfected with the plasmid, and transfection efficiency was assessed by measuring the luciferase activity using the ONE-Glo™ Luciferase Assay System (n = 6).

SUMMARY

The PureYield™ Plasmid Miniprep System gives researchers a new option to prepare 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 with 0.6 ml of bacterial cell culture or harvested cells from up to 3.0 ml of bacterial cell culture.

PROTOCOLS

- *PureYield™ Plasmid Miniprep System Technical Bulletin*, #TB374, Promega Corporation
  www.promega.com/tbs/tb374/tb374.html

ORDERING INFORMATION

Product	Size	Cat.#
PureYield™ Plasmid Miniprep System	50 preps	A1221
	250 preps	A1222

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