High-Yield Plasmid DNA Purification Using PureYield™ Plasmid Miniprep System

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Materials

JM109 High Efficiency Competent Cells (Cat.# L2001)
Single-Use Pro 5-alpha Competent Cells (Cat.# L1221)
Terrific Broth
LB Medium
BamHI Restriction Enzyme (Cat.# R6021)
PureYield™ Plasmid Miniprep System (Cat.# A1223)
Diamond™ Nucleic Acid Dye (Cat.# H1181)
pBR322 Vector (Cat.# D1511)
pF3A WG (BYDV) Flexi® Vector (Cat.# L5671)
pGL4.10[*luc2*] Vector (Cat.# E6651)

Sample Volumes

50µl of each transformation was plated
1ng of DNA per transformation
0.6ml or 1.5ml of culture used in DNA isolation

Protocols:

- *PureYield™ Plasmid Miniprep System Technical Bulletin*, #TB374
- *Diamond™ Nucleic Acid Dye Technical Manual*, #TM388

Introduction

This article describes a method for purifying large quantities of plasmid DNA using the PureYield™ Plasmid Miniprep System. Transformed cells grown in enriched medium such as Terrific Broth produce significantly more plasmid using the PureYield™ Plasmid Miniprep System than when grown in standard LB medium.

Methods

JM109 High Efficiency Competent Cells and Single-Use Pro 5-alpha Competent Cells were transformed with three vector types (Table 1); 1ng of DNA was used per transformation. All three vector types contain a unique BamHI restriction site.

Table 1. Specifications of the Vectors Used.

Vector	Relative Copy Number	Size (bp)
pBR322	Low (15–20)	4,361
Flexi® Vector	Mid-High	3,981
pGL4.10[*luc2*]	High	4,242

Fifty microliters (50µl) of each transformation was plated on LB plates with ampicillin (100µg/ml) for 18 hours at 37°C. One colony from each plate was used to inoculate 50ml of Terrific Broth or LB Medium with ampicillin overnight at 37°C. For each sample, 0.6ml or 1.5ml of culture was removed and centrifuged for 3 minutes to form a cell pellet. The supernatant was removed, and all cell pellets were resuspended in 600µl of TE buffer prior to assaying. Plasmid DNA was isolated with the PureYield™ Plasmid Miniprep System according to the protocol in the *PureYield™ Plasmid Miniprep System Technical Bulletin*, #TB374. The concentration and purity of isolated plasmid DNA was determined by use of the NanoDrop® 1000 spectrophotometer.

Three hundred nanograms (300ng) of each sample was linearized using BamHI at 37°C for 1.5 hours in 20µl reactions. Ten microliters (10µl) of each digest was run on an 0.8% agarose gel and stained with Diamond™ Nucleic Acid Dye to confirm plasmid size.

Cell cultures grown in Terrific Broth reached approximately two times the cell density as those grown in LB Medium, although actual density depended on plasmid type (Figure 1).

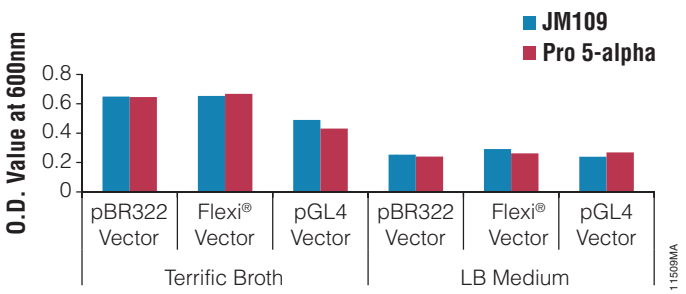

Figure 1. Absorbance values for JM109 and Single-Use Pro 5-alpha Competent Cells transformed with pBR322, a Flexi® Vector or pGL4.10[*luc2*] plasmids. Cells were transformed with plasmids in either Terrific Broth or LB Medium, with 100µg/ml ampicillin.

Cells grown in Terrific Broth produced higher DNA yields than the corresponding cells in standard LB Medium regardless of cell or plasmid type (Figures 2, 3). In many cases, processing 0.6ml of cell culture in Terrific Broth resulted in higher DNA yields than processing 1.5ml of cell culture in LB Medium. Purity ratios determined by NanoDrop® 1000 varied by plasmid type but were not significantly impacted by volume processed or medium used (Table 2).

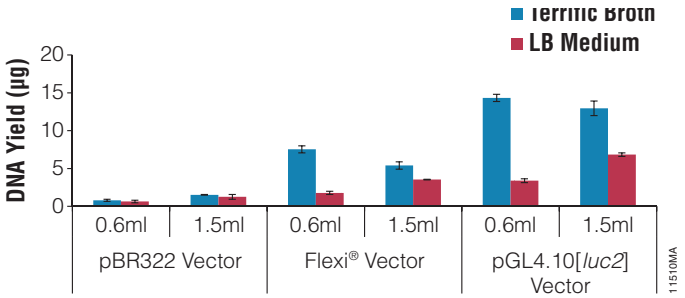

Figure 2. DNA yield from PureYield™ Plasmid Miniprep System for JM109 High Efficiency Competent Cells. Cells were grown in Terrific Broth or LB Medium with 100µg/ml ampicillin. The cells were transfected with pBR322, Flexi® Vector or pGL4.10[luc2] plasmids. All samples were analyzed in duplicate.

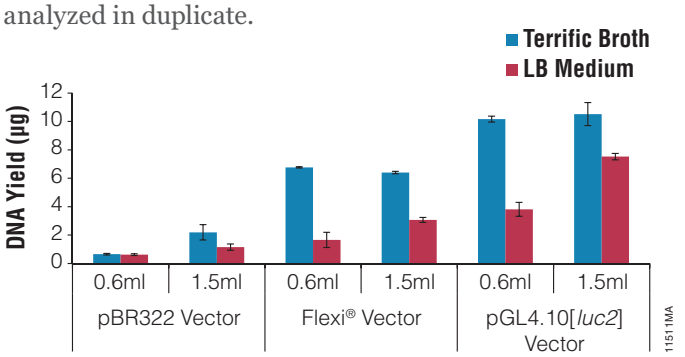

Figure 3. DNA yield from PureYield™ Plasmid Miniprep System for Single-Use Pro 5-alpha Competent Cells. Cells were grown in Terrific Broth or LB Medium with 100µg/ml ampicillin. The cells were transfected with pBR322, Flexi® Vector or pGL4.10[*luc2*] plasmids. All samples were analyzed in duplicate.

Table 2. NanoDrop® 1000 Purity Ratios for JM109 High Efficiency Competent Cells and Single-Use Pro 5-alpha Competent Cells in Terrific Broth or LB Medium. Samples were processed in duplicate.

			JM109 High Efficiency		Pro 5-alpha	
			A_{260}/A_{280}	A_{260}/A_{230}	A_{260}/A_{280}	A_{260}/A_{230}
Terrific Broth	pBR322	600µl	1.58	1.32	1.85	2.59
		1.5ml	1.67	1.20	1.75	1.39
	Flexi® Vector	600µl	1.87	2.18	1.86	2.46
		1.5ml	1.82	1.88	1.83	2.04
	pGL4.10	600µl	1.85	2.24	1.83	2.17
		1.5ml	1.83	2.11	1.83	1.96
LB Medium	pBR322	600µl	1.60	1.73	1.83	1.03
		1.5ml	1.74	1.87	1.83	1.60
	Flexi® Vector	600µl	1.76	1.59	1.83	1.41
		1.5ml	1.86	2.31	1.83	2.09
	pGL4.10	600µl	1.83	1.9	1.83	1.96
		1.5ml	1.84	2.01	1.83	2.22

When the DNA samples were linearized with BamHI, correctly-sized fragments were observed for all samples, indicating that PureYield™ Plasmid Miniprep System efficiently isolated plasmid DNA (Figure 4).

However, we observed that processing more than 1.5ml of cells grown in enriched medium might result in decreased yields, possibly because a very large amounts of biomass reduces performance of the PureYield™ Plasmid Miniprep System.

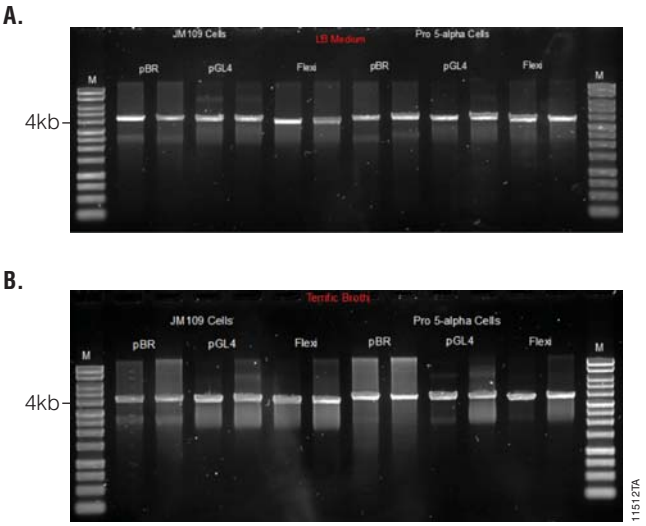

Figure 4. Plasmid DNA (150ng) was digested with BamHI at 37°C for 1.5 hours and analyzed on an 0.8% agarose gel stained with Diamond™ Nucleic Acid Dye. JM109 High Efficiency Competent Cells or Pro 5-alpha Competent Cells were transformed with pBR322, pGL4.10[*luc2*] or Flexi® Vector and cultured in LB Medium (**Panel A**) or Terrific Broth (**Panel B**). Lane M: Benchtop 1kb Ladder.

Conclusions

- Using the PureYield™ Plasmid Miniprep System, both transformed JM109 and Single-Use Pro 5-alpha Competent Cells produced higher DNA yields when grown in enriched medium compared to LB medium.
- The plasmid was of high quality when analyzed using gel electrophoresis and the NanoDrop® 1000.

Ordering Information

Product	Size	Cat.#
PureYield™ Plasmid Miniprep System	100 preps	A1223
	250 preps	A1222
Diamond™ Nucleic Acid Dye	500µl	H1181
BamHI Restriction Enzyme	2,500 units	R6021
pGL4.10[*luc2*] Vector	20µg	E6651
pBR322 Vector	10µg	D1511
pF3A WG (BYDV) Flexi® Vector	20µg	L5671
JM109 High-Efficiency Competent Cells	1ml	L2001
Single-Use Pro 5-alpha Competent Cells	1ml	L1221

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