

## Application Note

# Comparative Analysis of Media Types and Volumes for Plasmid Yield Optimization with the AmMag™ Quatro Mini 1100

Angela Huang<sup>1</sup>, Kamil Sekulski<sup>1</sup>, Janet Caverly<sup>1</sup>, Nilojan Jehanathan<sup>1</sup>, and Luciana Rosselli-Murai<sup>1</sup>

<sup>1</sup>Automated Protein & Plasmid Purification Technology Lab, GENSCRIPT USA INC, 20 Kingsbridge Rd., Piscataway, NJ, 08854, USA

## Introduction

Plasmid DNA purification is a critical step in many molecular biology workflows, including gene expression, mutagenesis, and high-throughput screening applications. Traditionally, manual plasmid miniprep kits have been the method of choice, involving multiple centrifugation steps and a series of buffer additions for cell lysis, plasmid binding, washing, and elution. While effective, these manual workflows are labor intensive, time consuming, and prone to variability, especially when scaled across multiple samples or operators. These limitations can create bottlenecks in research pipelines, particularly in high-throughput environments such as antibody discovery and screening.

To address these challenges, automated purification systems have emerged as reliable alternatives. GenScript's AmMag™ Quatro Mini 1100 is a fully automated benchtop instrument designed for small-scale, high-throughput plasmid isolation. Leveraging alkaline lysis and magnetic bead-based purification, the AmMag™ Quatro Mini 1100 automates the entire process—from cell lysis to final elution—requiring minimal user intervention. Users simply load the consumables, reagents, and bacterial cultures, and the instrument delivers purified plasmids under two hours.

In this application note, the plasmid yield and purity obtained using the AmMag™ Quatro Mini 1100 are assessed to identify the optimal *E. coli* growth conditions. Additional factors such as shaker speed and orbital diameter, which also influence plasmid yield, are addressed in a separate application note. Performance is compared to a commonly used manual miniprep kit, following the manufacturer's standard protocol.

## Materials and Methods

### Transformation

The pcDNA3.1(+) and pET28a(+) plasmids (GenScript) containing an ampicillin and kanamycin resistance gene, respectively, were transformed into DH5α competent *E. coli* cells according to the manufacturer's protocol. The transformed cells were plated on an LB agar plate with 100 µg/mL ampicillin or 50 µg/mL kanamycin and incubated at 37°C for 16 h.

### Bacterial Culture

LB, TB, and Plasmid+ (Thomson Instrument Company, Cat. No. 446300) media with appropriate antibiotic were aliquoted into 48-well plates to prepare cultures in quadruplicate. A single colony was picked from the plate to inoculate each well. The plate was sealed with a breathable film and incubated at 37°C and 800 rpm in a 3 mm orbital shaker (INFORS HT Multitron Incubator Shaker) for 16 h.

Bacterial cultures for manual plasmid purification were grown in tubes. 5 mL LB media with 100 µg/mL ampicillin or 50 µg/mL kanamycin were aliquoted into test tubes. A single colony was picked from the plate to inoculate each tube. The tubes were incubated at 37°C and 220 rpm in a 25 mm orbital shaker (Eppendorf Innova® 40, Cat. No. M1299-0090) for 16 h.

Bacterial cultures were harvested at 4,120×g in a swinging bucket centrifuge (Beckman Coulter Allegra X-14R) for 10 min at 4°C. Supernatant was removed and the pellets were stored at -20°C or processed immediately using the AmMag™ Quatro Mini 1100 or manual kits.

### DNA Purification

Plasmid DNA was purified from *E. coli* DH5 $\alpha$  cells carrying the pcDNA3.1(+) vector using both the AmMag™ Quatro Mini 1100 system and a QIAprep® Spin Miniprep Kit (Qiagen, Cat. No. 27106). For the automated workflow, the Low-Endotoxin Plasmid Purification Mini Kit (GenScript, Cat. No. L01037) and consumables (GenScript, Cat. No. D00056) was used in conjunction with the [AmMag™ Quatro Mini 1100](#) (GenScript, Cat. No. [D00043](#) for module, [D00055](#) for controller), following the protocols shown (Figure 1). The instrument module was set up according to standard operating procedures, with all consumables and reagents loaded prior to the automated run.

For comparison, plasmid purification using the manual miniprep kit was performed using traditional centrifugation-based methods, following the manufacturer's instructions.

Residual endotoxin levels were quantified using the Endosafe® nexgen-PTS™ system (Charles River, Cat. No. PTS150K). Each plasmid sample was diluted 1:100 (v/v) in LAL reagent water (GenScript) and loaded into 0.05 EU/mL Endosafe® LAL cartridges (Charles River, Cat. No. PTS5505F) in accordance with the manufacturer's instructions.

To verify structural integrity and quality, plasmid samples were subjected to restriction enzyme digestion using SmaI (BestEnzymes Biotech, Cat. No. EG15572S) and/or EcoRI (BestEnzymes Biotech, Cat. No. EG15536S), following the manufacturer's recommended protocol. Supercoiled, nicked, and linear forms were resolved on a 1.0% agarose gel run at 150 V for 20 minutes. Gels were stained with SuperStain (CoWin Biosciences, Cat. No. CW2635S), and band patterns were visualized and analyzed using the Image Lab software (Bio-Rad, Version 6.1).

**A**

Mix S1 Time: 300 s	S1 Vol: 185 $\mu$ L	WB2-1 Vol: 900 $\mu$ L
Mix S2 Time: 60 s	S2 Vol: 300 $\mu$ L	WB2-2 Vol: 600 $\mu$ L
S2 Standing Time: 240 s	S3 Vol: 300 $\mu$ L	EB Vol: 120 $\mu$ L
Mix S3 Time: 180 s	BD Vol: 700 $\mu$ L	Lysis Conditions: Medium
Binding Time: 600 s	EQ1 Vol: 150 $\mu$ L	Temperature: 65 $^{\circ}$ C
Mix WB2 Time: 120 s	EQ2 Vol: 60 $\mu$ L	<input checked="" type="checkbox"/> Temperature Control
Mix Elution: 300 s	ER Vol: 900 $\mu$ L	<input checked="" type="checkbox"/> Remove Endotoxin
Wait Elution: 300 s	WB1 Vol: 900 $\mu$ L	

**B**

Mix S1 Time: 300 s	S1 Vol: 277 $\mu$ L	WB2-1 Vol: 900 $\mu$ L
Mix S2 Time: 60 s	S2 Vol: 450 $\mu$ L	WB2-2 Vol: 600 $\mu$ L
S2 Standing Time: 240 s	S3 Vol: 450 $\mu$ L	EB Vol: 180 $\mu$ L
Mix S3 Time: 180 s	BD Vol: 700 $\mu$ L	Lysis Conditions: Medium
Binding Time: 600 s	EQ1 Vol: 150 $\mu$ L	Temperature: 65 $^{\circ}$ C
Mix WB2 Time: 120 s	EQ2 Vol: 90 $\mu$ L	<input checked="" type="checkbox"/> Temperature Control
Mix Elution: 300 s	ER Vol: 900 $\mu$ L	<input checked="" type="checkbox"/> Remove Endotoxin
Wait Elution: 300 s	WB1 Vol: 900 $\mu$ L	

**Figure 1. Programs used in the AmMag™ Quatro Mini 1100 to purify pcDNA3.1(+) plasmid.** (A) Protocol A was used for cells grown in LB medium. (B) Protocol B was used for cells grown in TB and Plasmid+ media. The main differences are the lysis conditions (i.e., volumes of S1, S2, and S3 buffers) to accommodate the larger pellet masses.

### Concentration, Purity, Quality, and Endotoxin Analysis

To assess plasmid yield and purity, 2  $\mu$ L of each purified pcDNA3.1(+) sample was analyzed using a NanoPhotometer® N50 (IMPLEN). Absorbance at 230 nm, 260 nm, and 280 nm was measured to assess DNA concentration and purity.

## Results and Discussion

### Optimization of Plasmid Yield in the Mini 1100

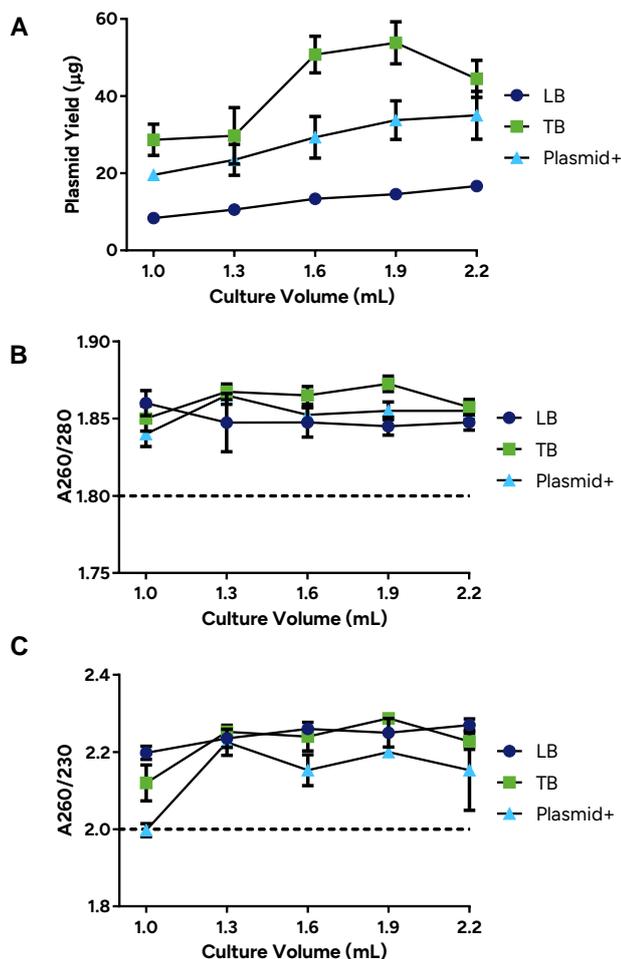
To maximize plasmid DNA yield using the AmMag™ Quatro Mini 1100, a series of optimizations were performed focusing on bacterial growth conditions in 48-well plates. *E. coli* DH5 $\alpha$  cells harboring the pcDNA3.1(+) plasmid were cultured in three different media types—LB, TB, and a proprietary Plasmid+ medium—at various volumes (1.0 to 2.2 mL) in a 48-deep well plate format.

TB media consistently produced the highest plasmid yields across all volumes, with yields peaking at 1.9 mL before declining slightly at 2.2 mL (Figure 2a). LB media produced the lowest plasmid yields, while Plasmid+ displayed a positive correlation between culture volume and yield. Based on these findings, TB at a 2.0 mL culture volume was identified as the optimal condition for plasmid production using the AmMag™ Quatro Mini 1100. Plasmid+ is a richer medium and is generally expected to produce higher yields. However, in this case, limited aeration—due to the culture volume and the type of plate used—restricted the yield obtained.

Plasmid purity was evaluated by measuring the A260/280 and A260/230 ratios (Figures 2b and 2c). All conditions yielded DNA of acceptable purity, as

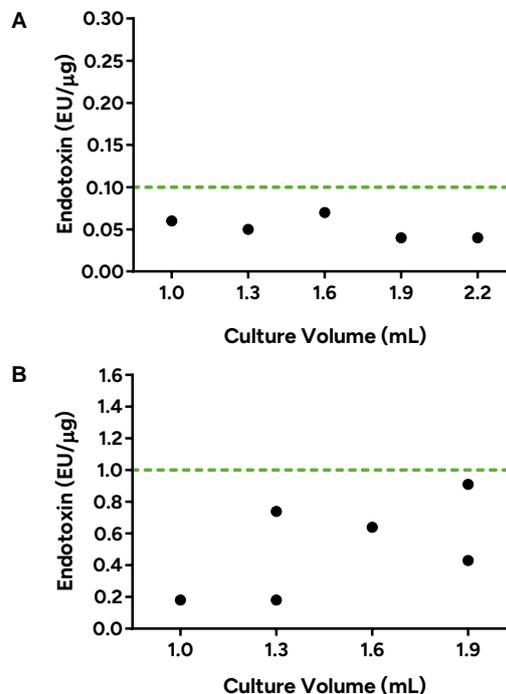
indicated by A260/280 ratios between 1.8 and 2.0 and A260/230 ratios above 2.0, reflecting minimal contamination from proteins, phenol, guanidine, or residual salts.

should be guided by both yield requirements and downstream application needs.



**Figure 2. Analysis pcDNA3.1(+) plasmid purified from pellets grown in different media and culture volumes using the AmMag™ Quatro Mini 1100.** (A) Yield of pcDNA3.1(+) plasmid with the TB media having the highest for all culture volumes. (B) A260/280 ratio and (C) A260/230 ratio of pcDNA3.1(+) purified plasmid

Plasmids purified from cells grown in LB medium consistently resulted in endotoxin levels below 0.1 EU/µg across all volumes (Figure 3A). For plasmids from cultures grown in TB medium, endotoxin levels were below 1 EU/µg (Figure 3B). Although TB media showed higher endotoxin levels compared to LB media, both media produced plasmid DNA suitable for transfection, with endotoxin levels below 1 EU/µg in mini prep scale. Therefore, selection of growth medium



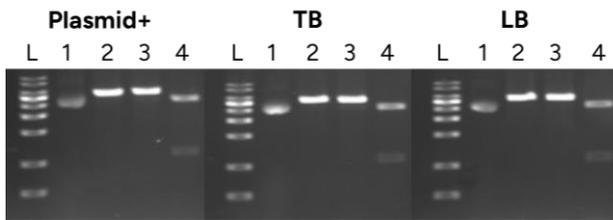
**Figure 3. Endotoxin concentrations of pcDNA3.1(+) plasmid samples purified from DH5α cells grown in different media.** (A) Cells grown in LB medium at all culture volumes had endotoxin levels below 0.1 EU/µg (green line). (B) Cells grown in TB medium had higher endotoxin levels, but still below 1 EU/µg (green line).

The structural integrity of the purified plasmids was verified by single and double digestions with EcoRI and SmaI, performed in triplicate for each medium. The resulting DNA fragments from one representative sample per condition were analyzed via agarose gel electrophoresis (Figure 4).

All samples showed greater than 95% supercoil ratio with negligible genomic DNA contamination. Plasmid samples were successfully linearized by single digestion with SmaI or EcoRI, and produced the expected fragment sizes upon double digestion, confirming the plasmid's structural fidelity.

Initial testing demonstrated that the AmMag™ Quatro Mini 1100 achieved high-copy plasmid yields of approximately 18 µg from cultures grown in LB, 38 µg from Plasmid+ medium, and up to 50 µg from TB. Further optimization of growth conditions—including media composition, culture parameters, and system protocols (detailed in a separate application note)—

significantly enhanced plasmid output. Under these refined conditions, yields increased to approximately 20  $\mu\text{g}$  with LB, 60  $\mu\text{g}$  with Plasmid+, and 80  $\mu\text{g}$  with TB.



**Figure 4. Agarose gel of pcDNA3.1(+) plasmid isolated from DH5 $\alpha$  cells grown in Plasmid+, TB, and LB media.** Each sample was loaded into a 1.0% agarose gel, ran at 150 V for 20 min, and visualized with SuperStain. L: 100 bp ladder; Lane 1: uncut plasmid; Lane 2: single digestion with SmaI; Lane 3: single digestion with EcoRI; Lane 4: double digestion with SmaI and EcoRI.

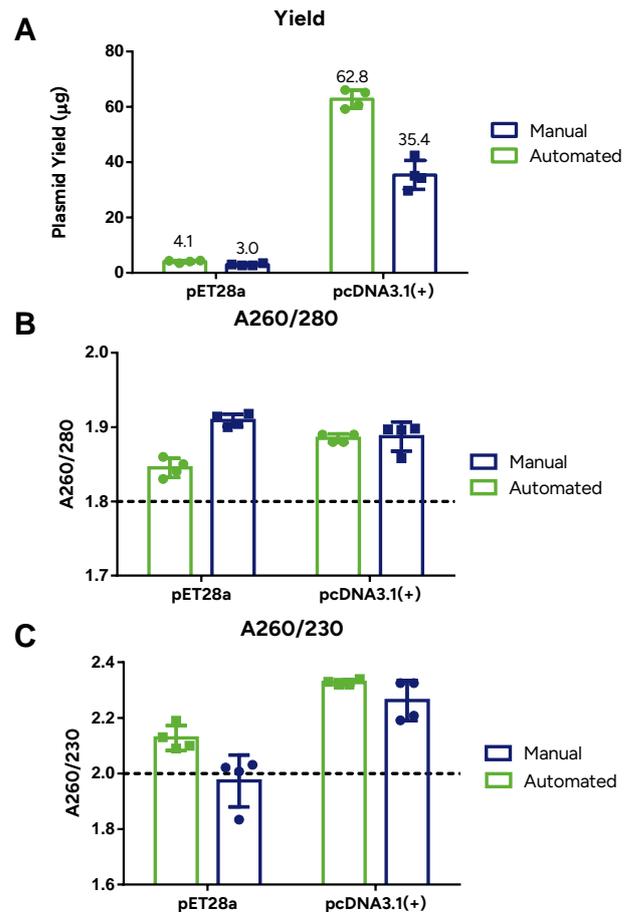
### Comparison to Manual Miniprep Plasmid Purification Kit

The performance of the AmMag™ Quatro Mini 1100 was benchmarked by comparing its plasmid purification efficiency and quality to a widely used silica membrane manual miniprep kit. *E. coli* DH5 $\alpha$  cells with either the high-copy pcDNA3.1(+) or low-copy pET28a plasmid were cultured under each manufacturer's recommended conditions. For the AmMag™ Quatro Mini 1100, cells were grown in 2 mL TB medium in a 48-well plate format, while for the manual miniprep, cells were grown in 5 mL LB medium in culture tubes. Plasmids were extracted using each system's standard protocol and analyzed accordingly (Figure 5).

For the low-copy pET28a plasmid, the AmMag™ Quatro Mini 1100 yielded 36.7% more DNA compared to the manual kit (Figure 5A). In addition to superior yield, plasmids purified using the AmMag™ Quatro Mini 1100 exhibited higher A260/280 and A260/230 ratios, indicating higher purity (Figures 5B and 5C). Notably, the lower ratios observed in manual prep samples may be partially attributed to their lower DNA concentrations, which can skew absorbance-based purity measurements.

For pcDNA3.1(+), a high-copy plasmid, the AmMag™ Quatro Mini 1100 yielded 77.4% more plasmid DNA than the manual miniprep kit (Figure 5A). This substantial difference in yield is attributed to the higher binding capacity and scalability of the magnetic bead-based system used in the AmMag™ Quatro Mini 1100. Magnetic beads can bind up to 1  $\mu\text{g}$  of DNA per  $\mu\text{L}$ , with customizable volumes ranging from 10 to 200  $\mu\text{L}$  per sample. In contrast, traditional silica membrane

columns are limited to a fixed DNA binding capacity of approximately 20–30  $\mu\text{g}$  per miniprep. The purity of pcDNA3.1(+) plasmids—measured by A260/280 and A260/230 ratios—was comparable between the automated and manual methods (Figures 5B and 5C), indicating that both approaches produce high-quality plasmid DNA.



**Figure 5. Comparison of plasmid purification of pcDNA3.1(+) and pET28a(+) using the automated AmMag™ Quatro Mini 1100 and silica membrane manual kit.** (A) Yields of the purified plasmids. (B) A260/280 ratios of the purified plasmids. (C) A260/230 ratios of the purified plasmids.

## Conclusion

The AmMag™ Quatro Mini 1100 offers a fully-automated, high-throughput platform for small-scale plasmid purification, delivering significantly improved performance compared to conventional manual miniprep kits. For the AmMag™ Quatro Mini 1100, the ideal growth conditions were determined to be 2 mL TB media cultures incubated at 800 rpm and 37°C for 16 h in a 3 mm orbital shaker. The results are of a higher yield

and quality for both low- and high-copy plasmids compared to the manual miniprep kits.

## Summary

The AmMag™ Quatro Mini 1100 system allows scientists to fully automate the plasmid purification process and streamline their downstream applications. Compared to manual miniprep methods, the AmMag™ Quatro Mini 1100 system offers (1) high yield, high quality, and ready-to-use plasmids, (2) scalability with the capacity to process 48 samples per module, (3) short runtimes that are under 2 h for all protocols, and (4) customizability with the ability to change lysis conditions, wash volumes, mixing times, magnetic beads volumes, and elution volumes.

## Ordering Information

<b>Product</b>	<b>Contents</b>	<b>Cat. No.</b>
AmMag™ Quatro 1100 System Controller	Controller that can connect up to 4 automation modules, simultaneously	D00055
AmMag™ Quatro 1100 Automation Purification Module	Automation Module	D00043
AmMag™ Quatro Plasmid Mini Consumables, 96 Preps	For 96 preps: sample plates, buffer tanks, reaction vessels, tips, collection plates	D00056
AmMag™ Quatro Low-Endotoxin Plasmid Purification Mini Kit	For 48-preps: buffers in individually packaged bottles	L01037-48