Application Note

Optimizing Plasmid Yield with the AmMag™ Quatro 1400 with Separation Elution Protocol

Nilojan Jehanathan[1], Janet Caverly[1], Guillermo Fernandez Rodriguez[1,2] and Luciana Rosselli-Murai[1]

[1]Automated Protein & Plasmid Purification Technology Lab, GENSCRIPT USA INC, 20 Kingsbridge Rd., Piscataway, NJ, 08854, USA

[2]GENSCRIPT BIOTECH (NETHERLANDS) B.V., Alexander Fleminglaan 1, 2613 AX Delft, Van Iterson House, Netherlands

Introduction

The AmMag™ Quatro 1400 is an advanced, fully automated solution for the purification of large-scale, high-quality, transfection-grade plasmid DNA from *Escherichia coli* cultures. Designed to meet the growing demands of high-throughput molecular biology workflows, the Quatro 1400 delivers consistent performance, minimal hands-on time, and reliable scalability for research and production environments.

The system features a centralized controller that enables the simultaneous operation of up to four independent purification modules that can process 24 samples at a time, allowing users to run multiple protocols in parallel for enhanced throughput and flexibility. Each run utilizes a pre-filled consumable kit, which includes all necessary components: sample tube, reaction tube, reagent cartridge, tip box (containing the elution tube), and waste container—streamlining setup and minimizing user error. Users only need to load the samples and consumables into the instrument. The system will automate all the steps to yield purified plasmids in ~ 2 hours.

The AmMag™ Quatro is optimized for magnetic bead-based plasmid purification, delivering high yields of DNA with purity levels suitable for sensitive downstream applications such as in vitro transcription (IVT), transfection, cloning, and sequencing.

In this application note, we analyze the effects of binding beads and elution volume titration for high-copy plasmids with separation elution method on yield, concentration, and quality of the plasmids.

Materials and Methods

Transformation

The pcDNA3.1(+) plasmid (GenScript) containing an ampicillin resistance gene was 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 and incubated at 37°C for 16 h.

Bacterial Culture

A single colony was picked from the plate and inoculated into 5mL started culture containing LB medium supplemented with 100 µg/mL ampicillin. The culture was grown at 37°C with shaking at 220 rpm for 8 hours in a 25 mm orbital shaker (Eppendorf Innova® 40).

Next, 500 µL of the starter culture was inoculated into 500 mL of Terrific Broth (TB) medium, containing 100 µg/mL ampicillin, in a 2 L culture flask. The flasks were incubated at 37°C with shaking at 220 rpm for 16 hours in a 25 mm orbital shaker (Eppendorf Innova® 40).

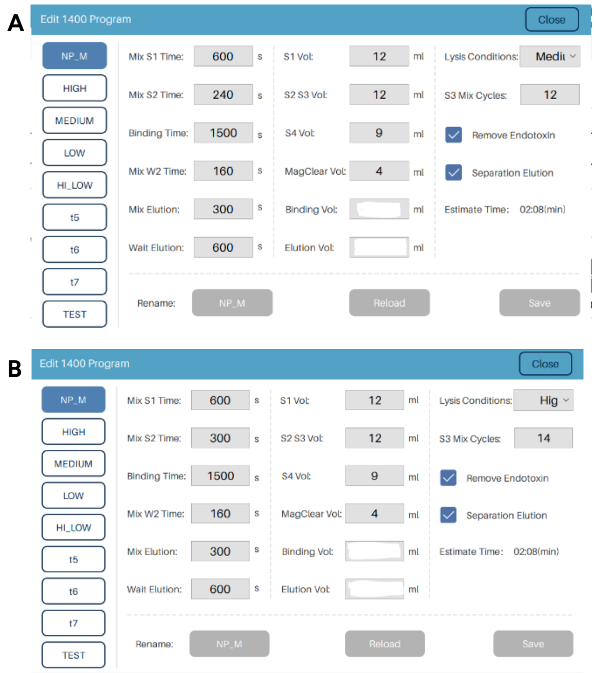
Pellet preparation

To harvest the cells, the resulting culture was combined and distributed into Quatro sample collection tubes, each containing approximately 40 mL. The tubes were centrifuged at 4,000 × g for 15 minutes at 4°C. After centrifugation, the cell pellets were weighed and adjusted to 0.8–1 g for "Medium" pellets and 1–1.5 g for "High" pellets by centrifuging additional culture volume into the same tubes, before being frozen and stored at –20°C for further use.

GenScript Scripting Possibilities

DNA Purification

Purification of the pcDNA3.1(+) plasmid was performed using the AmMag™ Quatro 1400 with the standard kit (GenScript L00882-24), following the "Medium"



protocol for medium pellets and the "High" protocol for high pellets with "Separation elution" method (Figure 1), according to the manufacturer's instructions.

Figure 1. Programs used in the AmMag™ Quatro 1400 to purify pcDNA3.1(+) plasmid. (A) Protocol "Medium" was used for medium pellets. (B) Protocol "High" was used for high pellets. The main differences are the lysis conditions. Binding beads and elution volume were systematically varied keeping the ratio (1:1).

Concentration, Purity, Quality, and Endotoxin Analysis

The concentration and purity of the purified pcDNA3.1(+) plasmid samples were determined with a NanoPhotometer® N50 (IMPLEN). Two µL of sample was loaded on the pedestal and the absorbance at 230, 260, and 280 nm was measured.

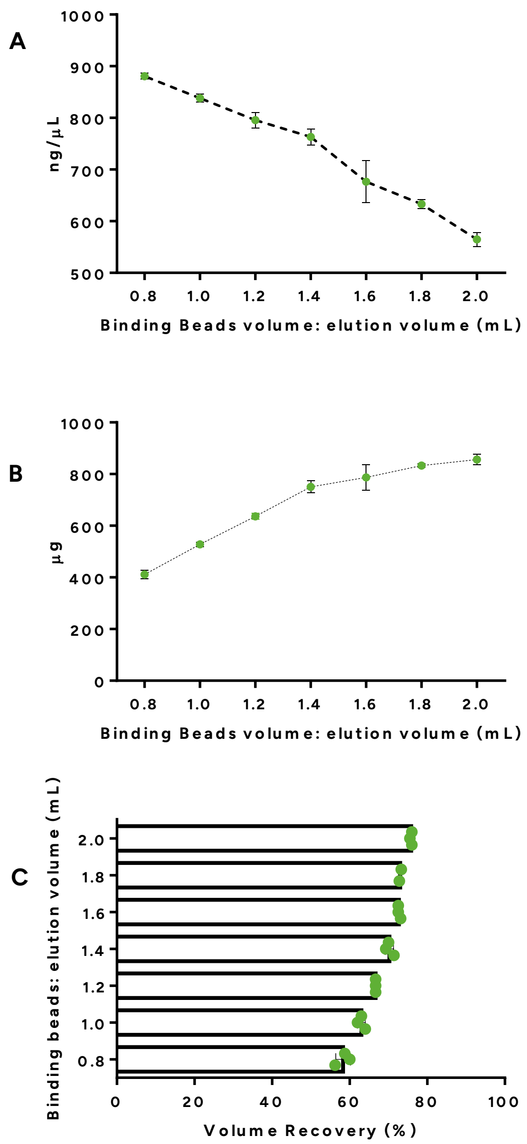
Endotoxin levels were measured using the Endosafe® nexgen-PTS™ (Charles River). Samples were diluted 1:100 mL/mL in LAL reagent water (GenScript) and loaded into Endosafe® LAL cartridges (Charles River).

Quality assurance was conducted using supercoil analysis and restriction enzyme digestion. Digestions were carried out with SmaI and/or EcoRI (New England Biolabs) according to the manufacturer's instructions. The resulting samples were loaded onto a 1.0% agarose gel, electrophoresed at 150 V for 20 minutes, and visualized under UV light. Supercoil analysis was performed using Image Lab software (Bio-Rad).

Results and Discussion

Optimization of Plasmid Yield with Binding Beads and Elution Volume Titration

In order to evaluate the effect of varying binding bead and elution volumes on key parameters such as plasmid yield, concentration, and volume recovery, the "Separation Elution" program was ran using medium-sized pellets (0.8 g to 1 g), as shown in Figure 2. Seven conditions were tested by proportionally varying the binding bead volume (0.6, 0.8, 1.0, 1.2, 1.4, 1.8, and



2.0mL) proportionally with the elution volume in three biological replicates.

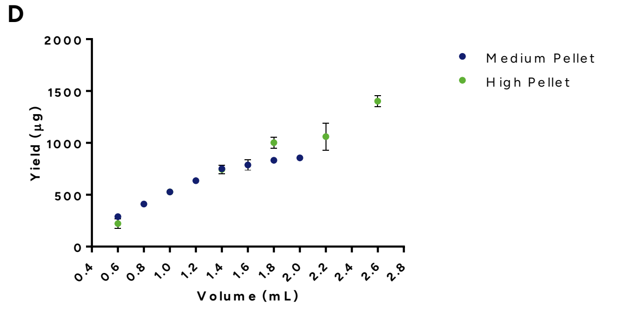


Figure 2. Titration of Binding Beads for High-Copy Plasmid Purification Using the AmMag™ Quatro with Separate Elution. (A) Scatter plot depicting the positive correlation between binding bead volume (mL) and pcDNA3.1(+) plasmid yield (µg). (B) Scatter plot illustrating the inverse relationship between binding bead volume and plasmid concentration (ng/µL). (C) Bar graph demonstrating the positive correlation between increasing binding bead and elution volumes and percentage volume recovery. (D) Correlation between plasmid yield (µg) and pellet size (medium vs. high). Scatter plot depicting the effect of binding bead-to-elution volume ratio titration on plasmid yield, with a positive correlation observed. Data are presented as mean ± standard deviation (SD). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparisons test; $P < 0.005$.

Results demonstrated a positive correlation between binding bead volume and plasmid yield. Yield increased proportionally with the binding bead and elution volumes, reaching a plateau at 1.8 mL, as shown in Figure 2B. As expected, higher bead/elution volume ratios led to a decrease in plasmid concentration due to increased dilution in the elution buffer, thereby lowering the concentration (Figure 2C). The effect of varying bead and elution volumes on volume recovery was also assessed (Figure 2D) and the results indicate that volume recovery percentage increased with higher bead/elution volume ratios. The percentage of volume recovery was calculated as the final eluted volume divided by the set elution volume, multiplied by 100, to account for losses during eluate transfer (bead retention) and final centrifugation (filter retention).

The effect of binding bead titration on plasmid yield was assessed using medium (0.8–1.0 g) and high (1.0–1.5 g) pellet weights (Figure 2D). A positive correlation between binding bead/elution volume and plasmid yield was observed for both pellet sizes. In medium pellet samples, plasmid yield plateaued at 1.8 mL of binding beads, suggesting that additional beads beyond this volume did not improve recovery due to limited DNA availability. In contrast, high pellet samples showed continued increases in yield beyond 1.8 mL, indicating that larger biomass contains more plasmid DNA that can be captured with additional beads. These results suggest that 1.8 mL of binding beads is sufficient for medium pellets, while higher volumes may be beneficial for larger biomass inputs.

Quality Analysis of the Purified Plasmids

To evaluate the impact of varying binding bead and elution volumes on plasmid quality, multiple QC metrics were assessed across different conditions using high-pellet samples of a high-copy plasmid.

Purity measurements, including A260/280 and A260/230 ratios, ranged from 1.8 to 2.0 and 2.0 to 2.2, respectively, indicating minimal contamination from proteins or salts. All samples exhibited supercoiled plasmid content greater than 80%, demonstrating high structural integrity in all conditions tested (Table 1).

Agarose gel electrophoresis further confirmed plasmid integrity (Figure 3A). Non-digested samples displayed a predominant supercoiled conformation with no detectable genomic DNA contamination. Restriction digestions using EcoRI and SmaI produced bands of expected sizes, supporting the absence of structural anomalies. Densitometric analysis of gel images (Figure 3B) showed no significant correlation between supercoiled content and binding bead volume, suggesting that supercoiling is maintained regardless of bead input. Endotoxin levels remained below 0.1 EU/µg for all conditions tested (Figure 3C).

The results indicated no significant correlation between the quality of plasmid DNA and the variation in binding bead and elution volumes. All tested conditions yielded high-quality plasmid preparations, demonstrating consistent purity, integrity, and low endotoxin levels across the range of volumes evaluated.

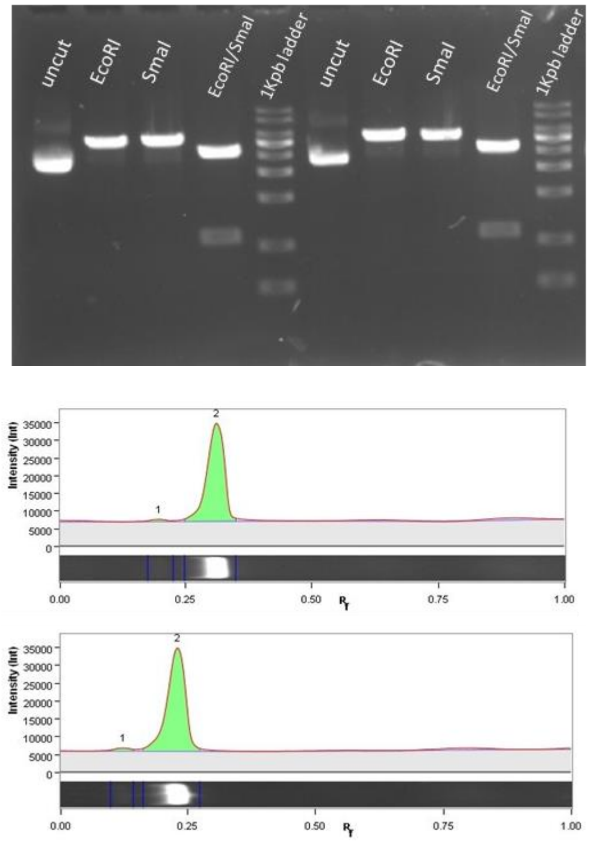


Figure 3. Quality analysis of the purified plasmids with AmMag™ Quatro. A) Agarose gel electrophoresis analysis of plasmid quality. Plasmid integrity was confirmed by Restriction Enzyme digestion with EcoRI and SmaI. B) Quantitative analysis of supercoil ratio was determined by densitometric analysis of the bands corresponding to the supercoiled and relaxed forms of plasmid DNA.

Conclusion

The AmMag™ Quatro 1400 provides a fully automated, high-throughput solution for plasmid purification. In addition to its streamlined workflow, the system allows users to optimize key parameters—such as yield and concentration—based on specific experimental needs, without compromising plasmid quality.

Summary

The AmMag™ Quatro 1400 system enables fully automated plasmid DNA purification while offering flexibility to optimize yield and concentration through adjustable binding bead and elution volumes. This adaptability supports a range of application needs without compromising plasmid quality.

Key findings include:

- Plasmid quality remains consistent across varying binding bead and elution volumes, with no adverse effects on purity, supercoiling, or endotoxin levels.
- Increased pellet mass (up to 1.5 g) correlates with higher plasmid yield.
- Higher binding bead and elution volumes improve total yield but result in lower DNA concentrations due to dilution effects.
- Conversely, lower bead/elution volumes produce more concentrated plasmid preparations, albeit with reduced overall yield

Note: Increasing the pellet weight beyond 1.5g would results in lower yield and quality due to incomplete lysis

Tables

Sample	Binding beads vol : Elution volume (mL)	A260/280	A260/230	Supercoil (%)	gDNA	Endotoxin levels (EU/ μg)
1	2.0	1.84	2.08	88.40	Negligible	< 0.1
2	1.8	1.89	1.92	88.50	Negligible	< 0.1
3	1.6	1.89	2.15	87.50	Negligible	< 0.1
4	1.4	1.9	2.13	87.00	Negligible	< 0.1
5	1.2	1.9	2.14	86.40	Negligible	< 0.1
6	1.0	1.89	2.13	87.70	Negligible	< 0.1
7	0.8	1.89	2.11	88.20	Negligible	< 0.1

Table 1. Quality assessment of selected plasmid DNA samples purified using the AmMag™ Quatro system.
This table summarizes key quality control metrics for selected samples presented in Figure 1. Parameters include the binding bead-to-elution volume ratio, plasmid concentration (ng/μL), absorbance ratios (A260/280 and A260/230), final plasmid yield (μg), supercoiled plasmid content (expressed as a percentage of total plasmid DNA), presence or absence of genomic DNA (gDNA), and endotoxin levels (EU/μg). All endotoxin levels were below the acceptable threshold of 0.1 EU/μg.

Ordering Information

Product	Contents	Cat. No.
AmMag™ Quatro 1400 System Controller	Controller that can control up to 4 purification modules, simultaneously	D00018
AmMag™ Quatro 1400 Automation Purification Module	One module can purify up to 6 samples per run	D00019
AmMag™ Quatro Plasmid Purification Kit	For 24 preps: sample tube, reaction tube, reagent cartridge with water as the elution buffer, tip box (containing the elution tube), and waste container	L00882
AmMag™ Quatro Plasmid Purification Kit (TE)	For 24 preps: sample tube, reaction tube, reagent cartridge with TE as the elution buffer, tip box (containing the elution tube), and waste container	L00943