High-Throughput, Automated Large-Scale Plasmid Purification: Eliminating Manual Bottlenecks in the Therapeutic Pipeline

Shadie Nimri, Lee Hoang, and Chris Suh
Biotage, 2350 Bering Dr., San Jose California 95131, USA

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Introduction

The rapid expansion of biologics discovery – from high-throughput antibody screening to the development of viral vectors for gene therapy – has created an unprecedented demand for high-quality, transfection-ready plasmid DNA at scale. Traditional large-scale purification methods (Maxiprep, Megaprep, and Gigaprep) remain heavily dependent on manual, gravity-flow protocols and labor-intensive alcohol precipitation. These "legacy" workflows are not only time-consuming but are prone to human error and inter-operator variability, often resulting in inconsistent DNA concentrations and yields that can stall downstream transfection and expression studies.

To address these challenges, we introduced Biotage® PhyPrep, the first fully automated platform designed to manage the entire large-scale purification workflow from cell lysis to final concentration. By utilizing proprietary dual-flow chromatography technology within a closed, automated system, the PhyPrep ensures maximal binding efficiency and with >80% supercoiled plasmid DNA.

A critical advancement of this system is the integrated on-column concentration kit, which automates the traditional manual alcohol precipitation step. This allows for the direct elution of highly concentrated DNA with significantly reduced hands-on time. In this poster, we demonstrate how the PhyPrep provides a scalable, "walk-away" solution that delivers superior reproducibility, exceptional purity, and high-concentration yields required for the most demanding modern bioprocessing applications.

Biotage PhyPrep

Biotage PhyPrep delivers consistent high-purity plasmid DNA at Maxi, Mega, and Giga scale (1, 5, 10 mg) with reliable results.

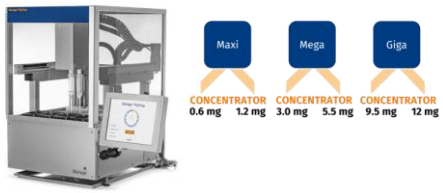


Figure 1. *Biotage PhyPrep system and concentrator column capacity and selection.*

Following Maxi, Mega, and Giga purification, users have the option to select appropriate concentrator columns to maximize yield and concentration.

High yield and high concentration

The range of concentrator columns allows users to tailor their workflow by selecting a column that optimizes either yield or final concentration, depending on the ideal elution volume for their downstream application. This flexibility is illustrated below, where identical samples were processed using both the high-yield and high-concentration columns. Together, these results highlight the system's versatility in delivering both exceptionally high yields and high-concentration eluates.

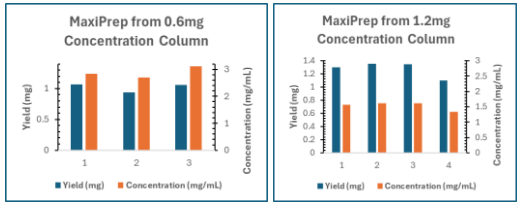


Figure 3. *Samples were processed as described. Three replicates of 2g cell pellet wet weight were processed using the MaxiPrep kit with both the 0.6mg or 1.2mg Concentration Columns. Yields are reported (A, B) along with the concentration of the final sample (C,D).*

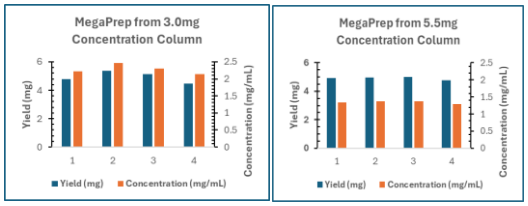


Figure 4. *Samples were processed as described. Four replicates of 4.5g cell pellet wet weight were processed using the MegaPrep kit and 3.0mg Concentration Column or 5.5mg Concentration Column. Yields are reported (A, B) along with the concentration of the final sample (C,D).*

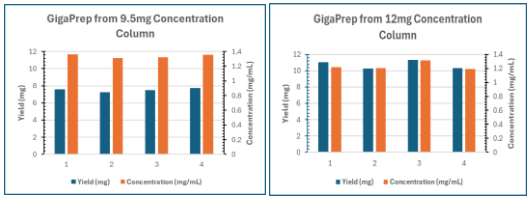


Figure 5. *Samples were processed as described. Four replicates of 7g cell pellet wet weight were processed using the GigaPrep kit and 9.5mg Concentration Column or 12.0mg Concentration Column. Yields are reported (A, B) along with the concentration of the final sample (C,D).*

Predictable concentration based on mass input

The concentrator columns deliver consistent plasmid DNA concentration regardless of upstream expression variables, including cell type, plasmid construct, or growth conditions. When combined with the flexibility of the PhyPrep concentrator system, researchers can reliably fine-tune their purification strategy to achieve the optimal plasmid quality for their workflow.

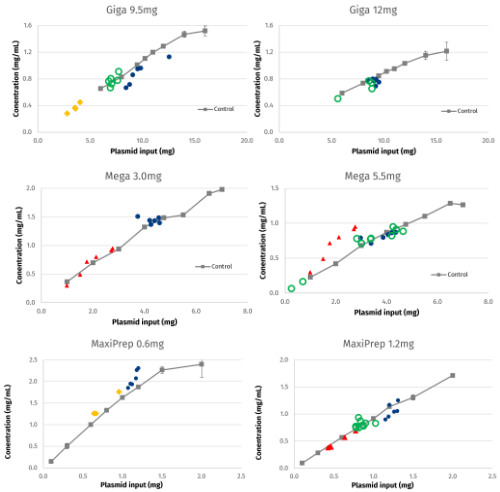


Figure 6. *Colored markers indicate data sets from different experimental cells, plasmids, and growth conditions*

Concentrated transient-transfection grade plasmid

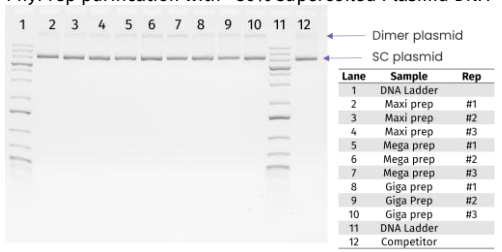
When paired with the highly selective PhyPrep purification columns, the concentrator produces plasmid DNA that surpasses transient-transfection quality requirements. This performance is demonstrated by transfecting mammalian Expi293 cells with a GFP plasmid and comparing the resulting expression levels between plasmids purified using the PhyPrep system and those obtained through manual purification.

Endotoxin (EU/µg) from replicate data

	MaxiPrep	MegaPrep	GigaPrep
1	0.003	0.003	0.010
2	0.003	0.004	0.005
3	0.005	0.004	0.024
4	0.008	0.004	0.005
5	0.004	0.004	0.010
6	0.004	0.005	0.011
7	0.005	0.004	0.009
8	0.002	0.005	0.005
9	0.005	0.005	0.005
10	0.005	0.006	0.007
11	0.005	0.006	0.006
12	0.006	0.005	0.006
13	0.005	0.005	0.009
14	0.010	0.007	0.006
15	0.005	0.008	0.027
16	0.005	0.007	0.060
Average	0.005	0.005	0.013
SD	0.002	0.001	0.014
CV	39	27	107

Figure 7. *16 replicates at each scale exceed endotoxin-free requirement*

PhyPrep purification with >80% supercoiled Plasmid DNA



Lane	Sample	Rep
1	DNA Ladder	
2	Maxi prep	#1
3	Maxi prep	#2
4	Maxi prep	#3
5	Mega prep	#1
6	Mega prep	#2
7	Mega prep	#3
8	Giga prep	#1
9	Giga Prep	#2
10	Giga prep	#3
11	DNA Ladder	
12	Competitor	

Figure 8. *Electrophoresis analysis of plasmid integrity shown on a 1% EtBr agarose gel of three Biotage® Maxi, Mega and Giga scale PhyPrep runs. All samples were normalized to 100ng of nucleotide per well from individual purification runs along with a leading competitor as a control in lane 12. The main product is supercoiled (SC) plasmid with the alternative plasmid dimer form.*

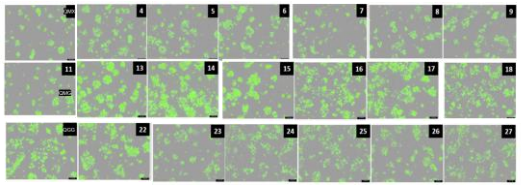


Figure 9. *Mammalian GFP Expression. Purified plasmids of GFP under the control of the CM promoter were used for transient transfection of expi 293 cells.* ***QMX****: Qiagen Maxi.* ***4-6****: PhyPrep 1.2mg Concentration column.* ***7-9*** *PhyPrep 0.6mg Concentration column.* ***QMG****: Qiagen Mega.* ***13-15****: PhyPrep 5.5mg Concentration column.* ***16-18*** *PhyPrep 3.0mg Concentration column.* ***QGG****: Qiagen Giga.* ***22-24****: PhyPrep 12mg Concentration column.* ***25-27*** *PhyPrep 9.5mg Concentration column.*

Summary

- **Complete Process Automation:** The Biotage® PhyPrep represents the first fully automated solution to manage the entire large-scale purification workflow—from cell lysis through final on-column concentration.
- **Elimination of Manual Bottlenecks:** By automating the traditionally labor-intensive manual purification and alcohol precipitation step, the system removes the primary source of human error and operator-to-operator variability.
- **Superior DNA Concentration:** Integrated dual-flow chromatography consistently delivers high-concentration eluates (**≥1 mg/mL**), providing transfection-ready plasmid DNA
- **Exceptional Sample Integrity:** Automated protocols ensure high yields of supercoiled DNA with low endotoxin levels, meeting the stringent purity requirements for sensitive cell-based applications.
- **Increased Lab Productivity:** Transitioning to a "walk-away" platform significantly reduces hands-on technician time, allowing researchers to focus on high-value downstream analysis and accelerating overall project timelines.
- **Scalable and Reproducible:** Whether at Maxi, Mega, or Giga scale, the PhyPrep provides a standardized, reliable method for producing high-quality plasmids.