Enhanced automated DNA purification with the CosMCPrep Kit on a Biomek i7 Workstation

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Introduction

Efficient and high-quality plasmid DNA (pDNA) extraction is critical for downstream applications such as transformation, transfection, sequencing and molecular cloning. The CosMCPrep Kit is designed to deliver high-yield and high-purity plasmid DNA from bacterial cultures. Through development of an automated CosMCPrep workflow on the Biomek i7 the dual Multichannel (MC) Workstation, we can significantly reduce hands-on time while ensuring reproducibility and scalability for micrograms to tens of milligrams scale plasmid DNA preps.

Objective

The aim of this paper is to describe the automation of the CosMCPrep Kit on the Biomek i7 Workstation to streamline scalable pDNA preparations with high efficiency by optimizing processing times and consumables and reducing user interventions when compared to a manual workflow. Our work demonstrates how the CosMCPrep Kit provides high yield plasmids with low endotoxin content when compared to kits from competitors—with the added advantage of achieving consistent yields of high-quality pDNA regardless of the adoption of a semi-automated or a manual setup.

Results and Discussion

We developed and tested an optimized protocol for pDNA production, based on our Beckman Coulter Life Science's patented SPRI paramagnetic bead technology, to achieve high-throughput pDNA preparation from *E. coli* cells.

The key enhancements in our revised protocol are:

1. Standardized ratio between processed biomass and magnetic beads, set at 0.25 g wet pellet per 80 µL of bead suspension
2. Incorporation of a filtration step to clean the neutralized lysate by filter plates
3. Use of Tris-EDTA (TE) buffer as eluent to improve performance in downstream DNA applications

While these optimizations are designed to scale up the process to achieve up to 10 mg or more of pDNA at Gigaprep scale, they have also proven highly effective for Mini-to-Maxi scale preparation. Moreover, in our experience, largely automating this workflow on a Biomek i7 workstation results in consistent and high-yield pDNA preps with minimal batch-to-batch variation (Figure 1) allowing for high reproducibility and time savings. Across all batch sizes (4, 24, and 96 samples), automation reduces hands-on time from 57 minutes (manual) to 15 minutes, a 74% reduction. This highlights the significant reduction in hands-on time achieved through automation, despite a longer total process time due to machine run-time. The data underscores the efficiency and scalability benefits of automation, particularly for high-throughput applications.

Table 1. Comparison of Manual vs. Automated Plasmid Preparation Workflows Across Varying Sample Sizes. The table outlines the time allocation, in minutes, for each step in the plasmid preparation process using manual and automated methods across three sample sizes (4, 24, and 96).

	4 Samples		24 Samples		96 Samples (Miniprep Only)	
	Manual	Auto	Manual	Auto	Manual	Auto
Aliquot reagents	7	6	7	7	7	4.5
Sample Resuspension	6	7.5	6	8.5	6	6
Sample Lysis	6	8.5	6	9.5	6	7
Sample Neutralizing	3	4.5	3	5.5	3	3
Sample Filtration	15	15	15	15	15	15
Sample Binding	14	31	14	33	14	29.5
Sample Washing	25	30.5	25	34	25	27
Sample Elution	26	31.5	26	33.5	26	28.5
Total Hands-On Time	57	15	57	15	57	15
Automation run time	NA	119.5	NA	131	NA	105.5
Total Process Time*	102	133.5	102	145	102	119.5

* Includes incubation and centrifuge times

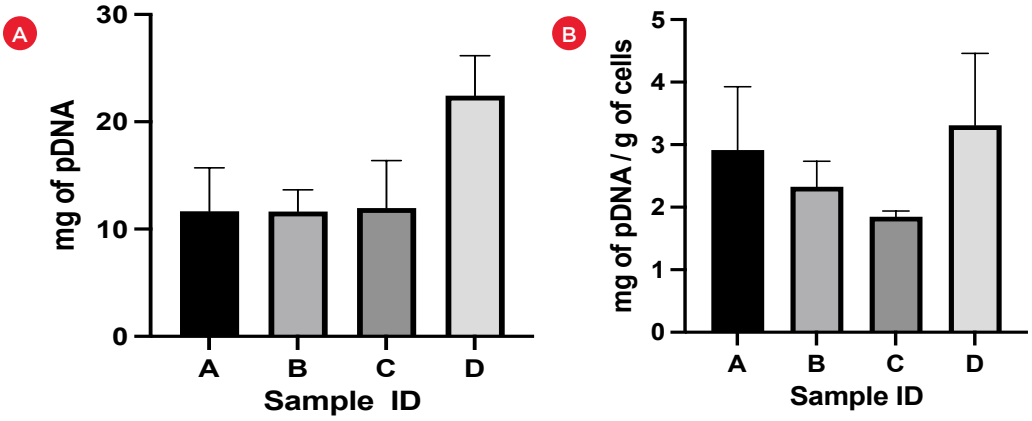
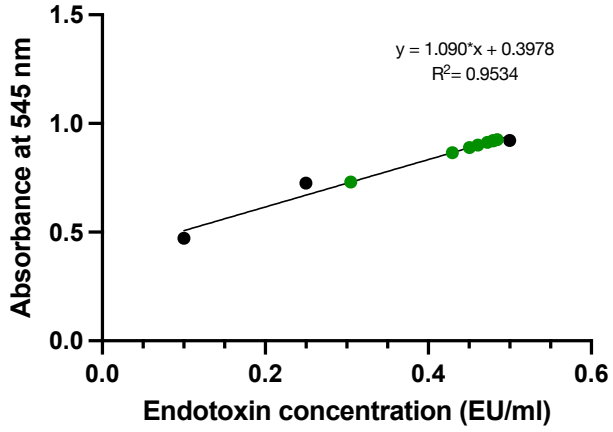


Figure 1: Representative yield of 4 Giga plasmid preps expressed as total prep yield (**A**) and yield normalized by grams of bacterial biomass processed in each prep (**B**). Plasmids A-D vary in size from 6.3 to 8.4 Kbp and originate from the same 6 Kbp parental vector. These vectors are used for gene expression in mammalian cells by transient transfections and contain a ColE1 ORI and an ampicillin resistance element (AmpR).

Crucially, this optimized protocol is performed in a semi-automated setup where the lysate clarification step is carried out by filtration without the support of automation. Although this might seem to be a redundant step that increases the overall costs of each pDNA prep, filtration is highly effective at removing flocs from the neutralized lysate, and eliminating the need for pelleting cell debris by centrifugation, which is inefficient for producing a compact pellet at 3500 x g for 30 minutes. Occasionally, softer pellets unpredictably detach from the tube/plate walls during clarified lysate transfer into a clean tube/plate prior to the DNA binding step. These instances result in heavy contamination, which irreparably undermines the final quality of the DNA prep without re-purification.

Standardization of the ratio biomass:beads as mentioned above is another key factor that allows this setup to deliver high-yield pDNA preps at scale with confidence. In our experiments, Gigapreps generated by this protocol provide a regular yield of 12 mg of pDNA, with some exceptional instances yielding as high as 20 mg of pDNA at the highest purity. Notably, for all tested samples, the endotoxin levels are found to be less than 0.1 EU/µg of pDNA (Table 2). This level is compatible with standard pDNA transfection procedures in mammalian cells, without requiring any additional endotoxin removal step (Figure 2). However, applying an endotoxin removal solution post-neutralization to achieve lower values than 0.1 EU/µg adversely affected yields without significantly reducing endotoxin levels (data not shown).



Prep ID	Interpolated values (EU/ml)	DNA Concentration (µg/ml)	Endotoxin concentration (EU/µg)
1	0.48	1554	$3.09*10^{-04}$
2	0.429	2272	$1.89*10^{-04}$
3	0.473	1920	$2.46*10^{-04}$
4	0.451	2345	$1.92*10^{-04}$
6	0.479	1995	$2.40*10^{-04}$
10	0.485	2869	$1.69*10^{-04}$
11	0.461	2793	$1.65*10^{-04}$
12	0.305	1929	$1.58*10^{-04}$

Figure 2 and Table 2: Endotoxin levels detection across a representative selection of preps. endotoxin concentrations are estimated as EU/mL (•) from the calibration curve shown above (•). These values are then converted into EU/µg DNA, as shown in the table 2. Endotoxin concentration of all samples considered in this ensemble is in the range of 10^{-4} EU/µg of DNA. Samples with < 0.1 EU endotoxins per µg of pDNA, are considered excellent for most transfection purposes. Interpolated Values, DNA Concentration, and Endotoxin Levels from Maxiprep Preparations (n=8).

Another key factor is the optimization of elution conditions, achieved by testing eluents at different pHs, including endotoxin-free Tris-EDTA (TE) at pH 7.5, endotoxin-free commercial elution buffer at pH above 9 and endotoxin-free water. Our data show that while pH variations of eluents impact marginally the DNA recovery from the beads, eluting the DNA in buffers with a pH far from neutrality can impair downstream sample applications, such as transfection of mammalian cells. Using pDNA eluted into endotoxin-free TE at pH 7.5, our results show a high transfection efficiency, with 90% of the cell population expressing a fluorescent marker 48 hours after the incubation with the transfection mixture (Figure 3).

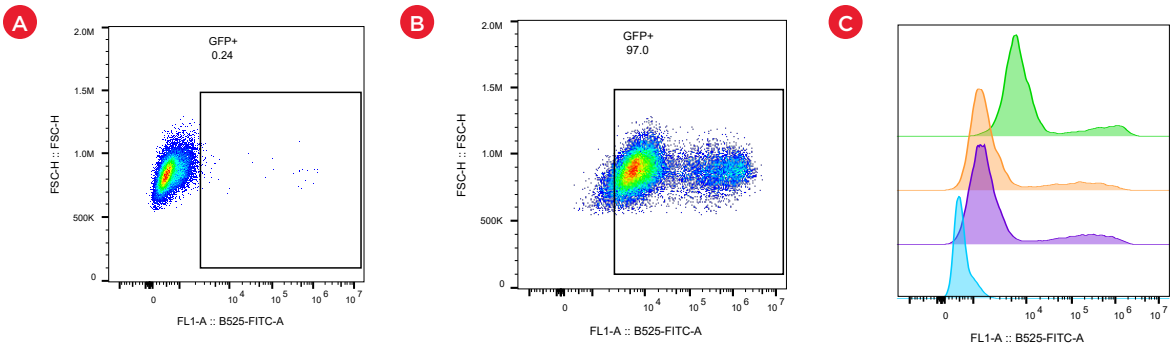


Figure 3: Transfection efficiency of sample A measured in Expi293 cells by FACS using the CytoFLEX flow cytometer (Beckman Coulter Life Sciences). **Panel A** displays the dot plot for the negative control, and **Panel B** shows the dot plot for cells transfected with the sample pDNA A, which leads to the expression of the GFP as positive transfection marker. The FACS analysis indicates that more than 90% of the cell population in Panel B express the fluorescent marker 48 hours post-transfection. The cell viability is around 90%, and the average cell diameter is 16.15 µm. **Panel C** highlights the increased fluorescent population observed when TE buffer (green) is used as eluent for the transfecting pDNA compared to water (purple) or a commercial elution buffer (orange). Negative control is shown in cyan.

Conversely, the same pDNA eluted by water (resulting in a pH 4 sample) or an alternative commercial elution buffer at pH > 9, leads to significant reduction of the transfection efficiency with < 45% green fluorescent protein (GFP) positive cells detected by FACS (Figure 3). Therefore, endotoxin-free TE at pH 7.5 is recommended as the preferred eluent in this protocol due to its superior performance in downstream application such as transfection, DNA editing and sequencing. Moreover, this optimized protocol outcompetes most protocols designed for commercial kits, which address the need of prepping pDNA in a TE-like buffer by recapturing the purified pDNA on beads, or precipitating it by 70% ethanol and resuspending it afterward as required. These additional steps often cause a loss of material and greatly inflate the overall length of the pDNA prep.

Remarkably, the CosMCPrep Kit yields consistent results regardless of whether it is carried out on an automated platform or manually using a multichannel pipette and magnetic plate (Figure 4). This feature makes the CosMCPrep Kit well-suited for small- to medium-sized labs seeking high-throughput pDNA preparation.

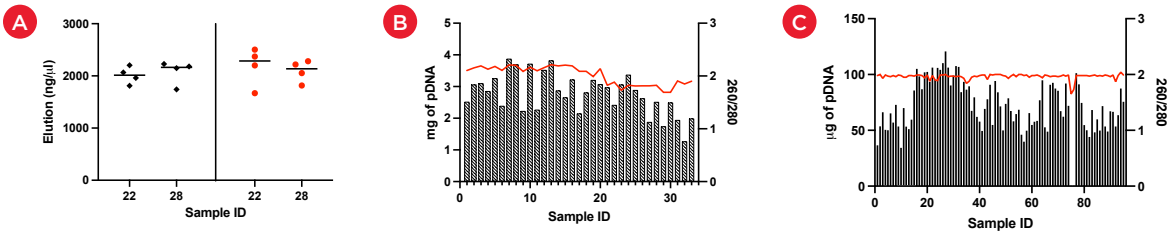


Figure 4: A- Comparison of prep yield for two plasmids (22 and 28), each of them prepped 4 times in the Midi format using the manual (♦) and automated (•) setup, respectively. **B**- Data from 33 Maxi preps plotted as mg of DNA/g wet pellet (grey histogram bars) overlaid with correspondent 260/280 values (–). **C**: Data from 96 Minipreps plotted as µg of DNA/1 mL liquid culture (grey histogram bars) overlaid with correspondent 260/280 values (–).

Table 3. Overview of scalability for the optimized CosMCPrep Prep protocol performed to generate micrograms to tens of milligrams of pDNA.

Prep Scale	Volume of beads (µL)	Average Yield (mg)	Average 260/280
Giga	1920	12	1.9
Maxi	320	2	2.0
Midi	80	0.5	1.8
Mini	10	0.007	1.9

Moreover, our results also demonstrate that scaling down this protocol from Giga to Maxi, Midi, or Mini preps does not compromise pDNA yields or quality in semi-automated and manual mode (Table 3, Figure 4) as also confirmed by by visual inspection on agarose gel and sequencing data (Figure 5).

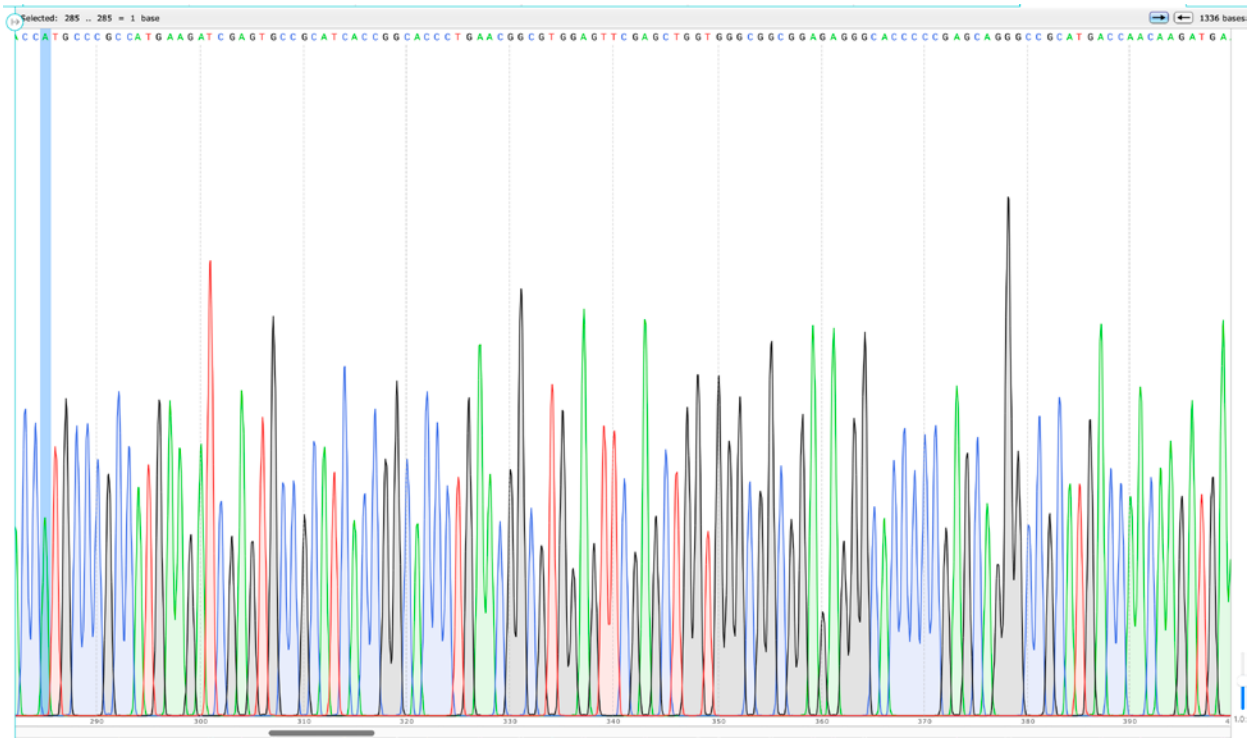


Figure 5: Snapshot of sequenced profile of the purified plasmid.

In our setup, automation significantly reduces hands-on time and costs, and improves overall throughput compared to traditional column-based extraction kits (Table 3). Moreover, purity and yield of samples produced in this setup consistently meet or exceed industry standards for high-quality DNA, even when comparison is drawn between this setup and equivalent competitor systems, as in the case of the fully automated platform X working with a proprietary pDNA extraction kit, or with a leading commercial manual Gigaprep kit, Y (Table 4).

Table 4. Comparing Gigaprep-optimized CosMCPrep process with direct competitor X and indirect competitor Y. Competitor X is a fully automated large scale purification system and competitor Y is a manual Giga-scale purification system commercially available worldwide.

Giga Scale	Beckman Coulter	Competitor X	Competitor Y
Timing (h)*	1.5-2.5	2	>3
Average yield (mg)/g of cells	2	0.26	0.83
Endotoxin level (EU/µg of DNA)	$1.39*10^{-4}$	$1.3*10^{-3}$	$2.5*10^{-4}$

*From cell pellet to DNA elution, see table 1.

Conclusion

Integration of the CosMCPrep Kit with a Biomek i7 Workstation enables efficient, scalable, and high-throughput pDNA preps. We demonstrate that our optimized protocol ensures high yield, purity and reproducibility, making it an ideal solution for laboratories requiring any scale of purified pDNA, from Mini to Gigapreps, even in the case of reduced or absent automation.

Contributions and Acknowledgments

P.A, L.S and S.V. initiated the project, later joined by M.S. P.A supervised the project. P.A, L.S and M.S designed and tested Mini-to-Giga automated workflows. P.A. and L.S performed automated and manual pDNA preps. PA and M.S optimized automated workflows. LS performed transfections and endotoxin assay. PA and LS equally contributed to the data collection. P.A. performed data analysis using GraphPad Prism, Version 10.4.1 for macOS, 2024, and wrote the manuscript. S.S and H.W contributed to the development and refinement of the application note's messaging framework, identified and requested essential data to support the content, and participated in brainstorming strategies for effective data presentation. All authors read, reviewed and approved the manuscript.

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Materials and Methods

Materials:

- CosMCPrep Prep Kit SKU A37064 or A29174, Beckman Coulter Life Sciences
- Biomek i7 Workstation, Beckman Coulter Life Sciences
- Standard microbiological equipment and consumables for growing bacteria (Innova shaker incubator or similar, 0.25 L and 2.5 L baffled flasks, selective antibiotics, selective agar plates, inoculating loops)
- Magnum FLX®24HV Universal 24-Well Magnet Plate SKU: A000640, Alpaqua
- 24-Well Blocks RB SKU 19583, Qiagen
- AcroPrep 24-well Cell Clarification and Sterile Filtration Plate-Depth filter plus 0.65/0.2 µm SuporEKV (8/pkg) SKU: 97026, PALL
- Reagent Grade Water, Ambion AM9932, or equivalent
- 100% Isopropanol, American Bioanalytical AB-07015, or equivalent
- 80% Ethanol (EtOH), American Bioanalytical AB-00138, or equivalent
- Plasmid Plus Media Plasmid+® Media SKU 446300, Thompson
- Centrifuge Allegra V15R equipped with VS2.5-96 Swinging-Bucket rotor, Beckman Coulter Life Sciences, or equivalent
- Mixmate Plate and Tube Mixer SKU 41103800, Eppendorf®, or equivalent, (Manual preps) or Inheco Thermoshake Classic SKU 7100146, or equivalent (Automated preps)
- Collection plate/tubes for DNA elution
- Stunner or Nanodrop for quantification purity assessment
- ToxinSensor™ Chromogenic LAL Endotoxin Assay Kit, SKU L00350, GenScript

Summary of Giga workflow:

1. Bacterial Culture Harvesting

- Centrifuge culture at 4000 x g at 4°C for 15 minutes to pellet 0.25 g of bacterial cells in each well of the 24-well block.
- Resuspend every 0.25g of pellet in 800 µL of RE1.

2. Cell Lysis

   - Add 800 µL of L2 and incubate at room temp for the recommended time.
   - Neutralize with 800 µL of N3.
   - Clarify lysate via filtration.

3. DNA Binding and Washing

   - Load cleared lysate onto 80 µL of CosMCPrep magnetic beads preactivated in isopropanol.
   - Perform automated binding and 3 washing steps to remove contaminants.

4. Elution

   - Elute purified pDNA using endotoxin-free TE elution buffer.
   - Collect DNA into designated elution tubes.

5. Quality Control and Yield Assessment

   - Measure DNA concentration and purity using Nanodrop or Stunner.
   - Perform agarose gel electrophoresis to verify integrity.
   - Assess endotoxin levels.

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