# Plasmid Purification with Silica Resin in 5 mL DDS IMCStips (MidiPure)

**Onohije Pogoson** • **Patrick Kates** • **B. Todd Mullis** • **L. Andrew Lee**

Integrated Micro-Chromatography Systems, Inc., Irmo, SC **Contact:** inquiries@imcstips.com

## INTRODUCTION

- Plasmid purification is essential for biotherapeutic development, gene therapy, and synthetic biology. Traditional spin column methods are labor-intensive, limited in scalability, and prone to resin saturation at higher cell densities.
- By automating the plasmid DNA (pDNA) purification process on the Dynamic Devices System (DDS) Lynx, using loosely-packed silica resin in MidiPure IMCStips®, we have optimized binding, washing, and elution steps to achieve higher yields and improved efficiency.
- This workflow eliminates centrifugation, reduces hands-on time, and processes larger volumes while maintaining pDNA quality comparable to spin columns. Up to 117 µg of pDNA can be recovered in under two hours with minimal intervention.

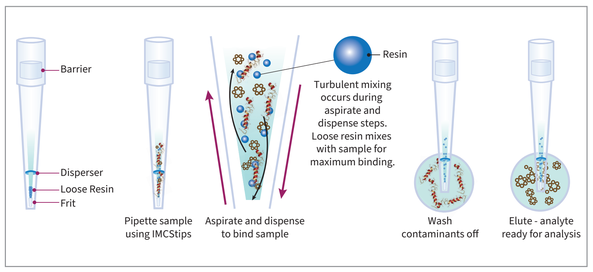


***Figure 1:*** *MidiPure IMCStips® containing loose resin employs dSPE to perform efficient automated extractions. Silica resins were used in IMCStips® to purify pDNA from bacteria cell lysate.*

## METHODS

- DH5α E. coli were transformed with plasmids of varying sizes: pCRS158 (8.5 kb), pPAK8K (8.0 kb), pCRS451 (6.2 kb), and pCRS240.4 (3.6 kb). Cultures were grown overnight at 37°C, 300 rpm.
- Bacterial cultures were pelleted via centrifugation and lysed via traditional alkaline lysis method. The cleared lysates were purified using 5 mL DDS IMCStips (MidiPure IMCStips®) preloaded with silica resin on the Dynamic Devices Lynx system.
- The purification process on the Lynx included resin preconditioning, sample binding, and a three-step alcohol-based wash to remove proteins, salts, and other contaminants.
- Purified pDNA was eluted in TE buffer and analyzed for yield and purity using a NanoDrop™. Sample integrity and usability for downstream applications was confirmed using agarose gel electrophoresis, Agilent TapeStation, and sequencing.

## RESULTS

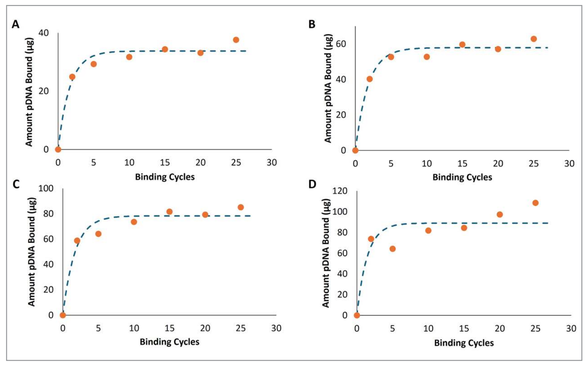


***Figure 2:*** *Langmuir adsorption isotherms for pDNA binding to varying amount (**(A)** 50 mg, **(B)** 100 mg, **(C)** 150 mg, and **(D)** 250 mg) of silica resin in 5 mL DDS IMCStips® across multiple binding cycles. The figure illustrates the relationship between the number of binding cycles and the amount of pDNA eluted. (dashed line: fitted data, circles: data collected).*

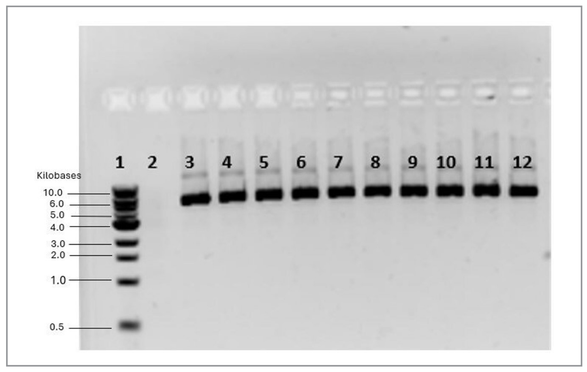


***Figure 5: Eluted plasmid DNA (pDNA) from varying cell densities ($OD_{600}$ = 20, 30, 50, 80, and 100).*** *The agarose gel shows the integrity of the purified pDNA across all $OD_{600}$ values with no signs of degradation or shearing. Lanes 3-4 represent pDNA extracted from 20 OD cultures, lanes 5-6 from 30 OD, lanes 7-8 from 50 OD, lanes 9-10 from 80 OD, and lanes 11-12 from 100 OD cultures. The ladder (1 kB) is positioned in lane 1 for molecular size reference.*

**Table 1.** Plasmid DNA Yield and Purity Across Different Plasmid Sizes

| Sample ID | Conc [ng/µL] | Yield [µg] | 260/280 | 260/230 | Q20/Len | Q40/Len |
|---|---|---|---|---|---|---|
| pCRS240.3 | 111 ± 4 | 56 ± 2 | 1.87 ± 0.01 | 2.04 ± 0.04 | 0.91 | 0.81 |
| pCRS451 | 115 ± 6 | 58 ± 3 | 1.88 ± 0.02 | 2.28 ± 0.04 | 0.96 | 0.85 |
| pPAK8K | 150 ± 5 | 90 ± 3 | 1.88 ± 0.01 | 2.13 ± 0.04 | 0.89 | 0.79 |

*pPAK8K – processed with 150 mg silica resin and eluted in 600 µL
*pCRS240.3 and pCRS451 – processed with 100 mg silica resin and eluted in 500 µL

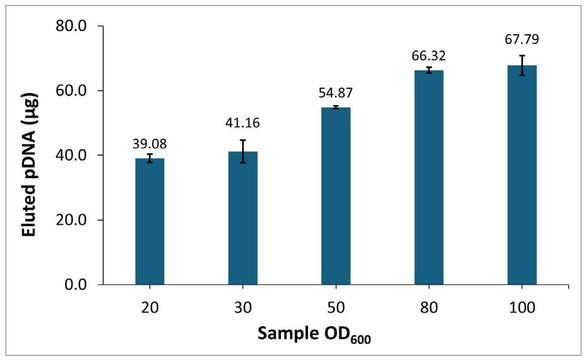


***Figure 3: Eluted pDNA Yield Across Different Cell Densities ($OD_{600}$).*** *Plasmid DNA yield (µg) was measured from bacterial cultures (pCRS451 (6.2 kb)) at varying OD levels (20, 30, 50, 80, and 100) using 100 mg silica for purification. Higher $OD_{600}$ values correspond with increased pDNA yield up to 80 OD, after which the gains plateau, suggesting that the resin's binding capacity is nearing saturation. Data shown are averages with standard error bars (n=4).*

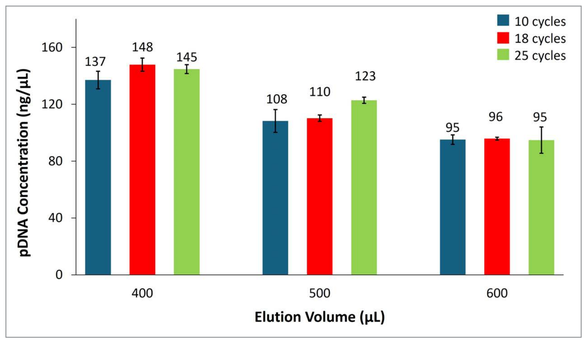


***Figure 6: pDNA concentration as a function of elution volume and aspiration-dispense cycle.*** *pCRS 158 (8484 bp) bacterial culture (50 OD per sample well) were purified using 100 mg of silica resin in MidiPure IMCStips®. Elution volumes of 400 µL, 500 µL, and 600 µL were tested across different cycles (● 10 cycles (≈12 minutes), ●18 cycles (≈22 minutes), ● 25 cycles (≈30 minutes)). The 400 µL volume produced the highest concentrations, while 500 µL ensured consistent resin coverage during elution while minimizing excessive dilution of the eluate. Data are presented mean ± standard error.*

**Table 2.** Comparative Analysis of pDNA Yield and Purity from the MidiPure IMCStips® (150 mg silica resin) and Midi Spin Column.

| Sample ID | $OD_{600}$ | ng/µL | µg | 260/280 | 260/230 |
|---|---|---|---|---|---|
| One tip (MidiPure IMCStips®) | 109 | 194.38 ± 6.33 | 116.6 ± 3.80 | 1.89 ± 0.01 | 2.06 ± 0.04 |
| Midi Spin Column | 205 | 418.73 ± 14.58 | 251.2 ± 8.75 | 1.89 ± 0.01 | 2.20 ± 0.01 |

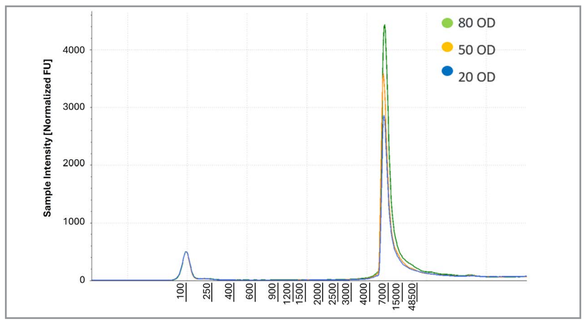


***Figure 4: Comparative electropherogram of eluted plasmid DNA (pDNA) from varying bacterial culture densities ($OD_{600}$ = 20, 50, 80).*** *The electropherogram shows a single, sharp peak for each sample, indicating the presence of intact, full-length plasmid DNA without signs of degradation or shearing. As cell density increases, the peak intensity also rises, reflecting higher plasmid concentrations at higher $OD_{600}$ values.*

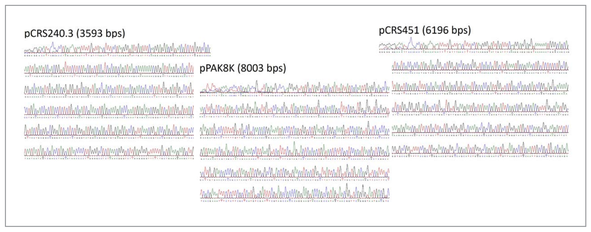


***Figure 7: Sequencing Electropherograms for Purified Plasmids.*** *Chromatograms representing the sequencing results for plasmids of varying sizes: pCRS240.3 (3.3 kb), pCRS451 (6.2 kb), and pPAK8K (8003 bp). The distinct and well-spaced peaks indicate the successful recovery of intact, high-quality plasmid DNA. Sequencing was performed to validate the integrity and usability of the purified plasmids, demonstrating consistent performance across different plasmid sizes.*

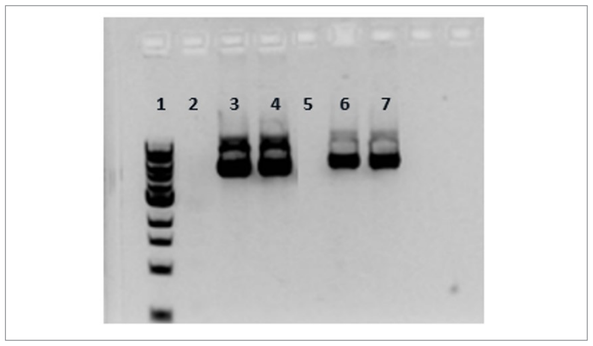


***Figure 8:*** *Comparison of tip-purified pDNA sample (5 mL IMCStips® packed with silica resin) to spin column purified pDNA. The figure shows 1 µL of eluted pDNA for the MidiPure IMCStips and Midi Spin column methods for pCRS158 plasmid (8484 bps). Lane 1: 1 kb DNA ladder; Lanes 3-4: Spin column; Lanes 6-7: MidiPure IMCStips®.*

## CONCLUSION

- The automated plasmid purification workflow developed for the Dynamic Devices System (DDS) Lynx utilizing MidiPure IMCStips® successfully addressed limitations of traditional spin column-based methods.
- Our optimized workflow achieved plasmid recoveries of up to 117 µg per tip with 150 mg silica resin, scaling efficiently to meet higher plasmid demands without compromising quality.
- Capable of processing 24 samples in 110 minutes, with excellent purity and integrity, validated through sequencing and gel analysis.
- This robust and scalable approach positions IMCStips® as a high-throughput solution for plasmid purification, advancing molecular biology workflows with minimal manual intervention.

## DOWNLOAD POSTER

Scan QR code to get access to this poster online.

imcs
integrated micro-chromatography systems