



An automated plasmid purification protocol – avoiding ancillary equipment and reducing manual intervention

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INTRODUCTION

- Plasmid purification plays a pivotal role in the development of new biotherapeutics, as well as in initial product development and enzyme engineering.
- Traditional plasmid purification methods require manual intervention and face limitations in throughput due to centrifuge space and balance requirements.
- This automated approach uses dispersive solid phase extraction (dSPE), eliminates the need for additional off-deck steps, and offers comparable yields and quality to traditional methods.
- Our method streamlines the purification process, allowing for between 1 and 96 samples to be purified in under one hour.

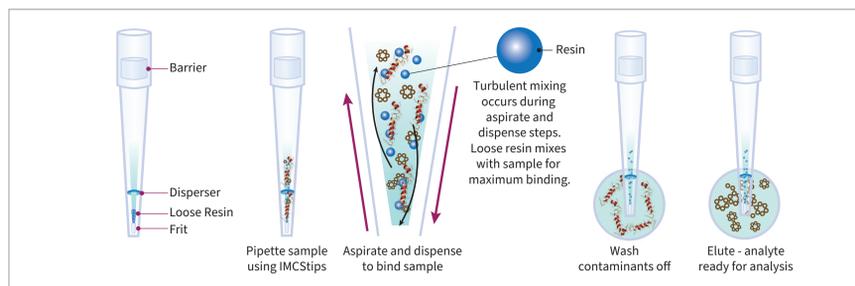


Figure 1: IMCStips® containing loose resin employ dSPE to perform efficient automated extractions. Silica resins was used in IMCStips® to purify pDNA from bacteria cell lysate.

METHOD

- Four plasmids of different sizes: pCRS158 (8484 bp), pCRS156 (5534 bp), pCRS492 (4105 bp), and pCRS 240.4 (3262 bp) in DH5a E. coli were used for this work. Cultures were grown overnight at 37°C, 300 rpm.
- Overnight cultures were pelleted via centrifugation (4000 xg) and lysed via traditional alkaline lysis.
- Cleared lysate was transferred to a 96-well plate for automated purification. This process utilized 1 mL IMCStips® containing 30 mg of silica resin on the Hamilton Microlab® STAR™ (ML STAR) system.
- The purification method comprised steps including preconditioning the resin, sample binding, a two-step alcohol-based washing protocol, and a frit wash to remove unbound materials and contaminants.
- The purified pDNA was eluted in TE buffer. Quantitative analysis was carried out using a NanoDrop™ 2000, and the integrity of the pDNA samples was qualitatively assessed via gel electrophoresis.
- IMCStips® were tested against kits from commercial vendors using pooled cultures and manufacturer's instructions.

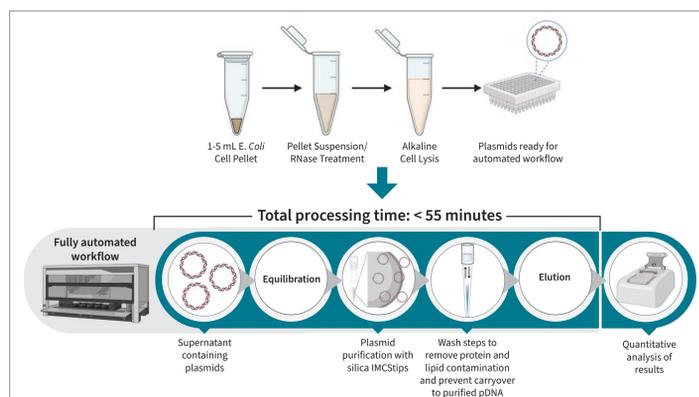


Figure 2. Overview of the plasmid purification workflow on the Hamilton ML STAR. Created with BioRender.com

INSTRUMENTATION AND DECK LAYOUT

- Hamilton Microlab ML STAR.

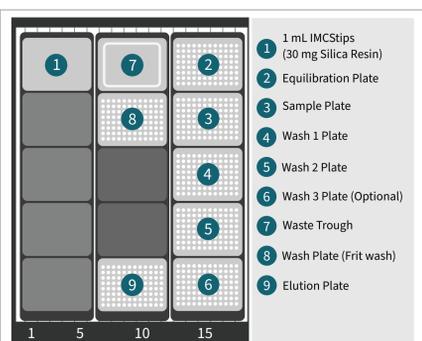


Figure 3. Deck layout of the automated plasmid purification on a Hamilton ML STAR.

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RESULTS

Comparing pDNA Yields and Purity Across Purification Kits

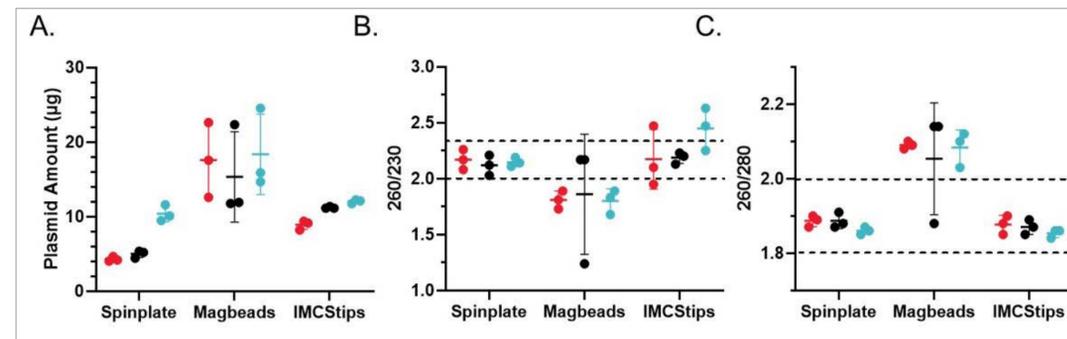


Figure 4. Comparative analysis of plasmid DNA purification methods across different plasmid sizes. A.) Total yield of pDNA (μg) isolated using three different purification methods: spin plate, magbeads, and IMCStips®. B.) Purity of pDNA as indicated by the 260/230 nm absorbance ratio. C.) Purity of pDNA as indicated by the 260/280 nm absorbance ratio. Dashed lines indicate optimal ranges. Data points are color-coded by the plasmid sizes (● 4k, ● 5k, ● 8k) used in the transformation. Error bars reflect the standard deviation.

Table 1: Comparative Analysis of pDNA Yield and Purity from Different Purification Methods Across Various Plasmid Sizes.

Method	Sample ID	Plasmid Size (bps)	pDNA (ng/ μL)	pDNA (μg)	260/280	260/230	Elution Volume (μL)
Spin Plate	4k	4105	28.60 \pm 1.70	4.29 \pm 0.26	1.89 \pm 0.0	2.17 \pm 0.1	150
	5k	5534	33.37 \pm 2.60	5.01 \pm 0.39	1.89 \pm 0.0	2.12 \pm 0.0	150
	8k	8484	69.23 \pm 5.90	10.39 \pm 0.88	1.86 \pm 0.0	2.15 \pm 0.0	150
IMCStips (30 mg Silica Resin)	4k	4105	59.43 \pm 3.30	8.92 \pm 0.50	1.88 \pm 0.0	2.17 \pm 0.2	150
	5k	5534	74.73 \pm 0.70	11.21 \pm 0.10	1.87 \pm 0.0	2.19 \pm 0.0	150
	8k	8484	80.27 \pm 1.40	12.04 \pm 0.22	1.85 \pm 0.0	2.45 \pm 0.2	150
Magbeads	4k	4105	439.60 \pm 102.3	17.58 \pm 4.09	2.09 \pm 0.0	1.81 \pm 0.1	40
	5k	5534	383.37 \pm 123.7	15.33 \pm 4.95	2.05 \pm 0.0	1.86 \pm 0.4	40
	8k	8484	459.07 \pm 110.1	18.36 \pm 4.40	2.08 \pm 0.0	1.80 \pm 0.1	40

- Yields and purities of spinplates and IMCStips® purifications were similar across plasmid size.
- Yields for magbead-based purifications were overreported by NanoDrop due to contaminants.
- 260/280 and 260/230 ratios for Magbead purifications were consistently outside expected range.
- Gel analysis showed similar yield, purity, and supercoiled content for spinplates and IMCStips®.

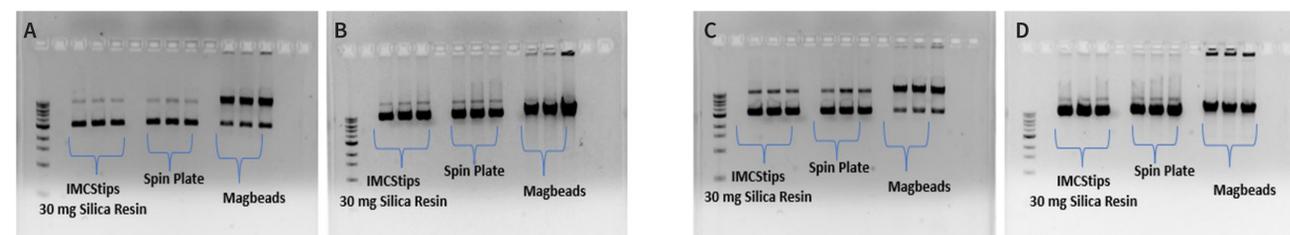


Figure 5. Comparison of tip-purified (1 mL IMCStips® packed with silica resin) normalize spin plate/spinplate and mag bead and magbead. Figures A and B show 1 μL of eluted pDNA for the IMCStips, Spin Plate, and Magbeads methods for two plasmid sizes (4k, and 8k). Figures C and D show normalized gel electrophoresis for direct comparison of purification methods, adjusting volumes to 3.75 μL for IMCStips and Spin Plate and maintaining 1 μL for Magbeads to equalize pDNA amounts.

Binding Capacity for Silica Resin

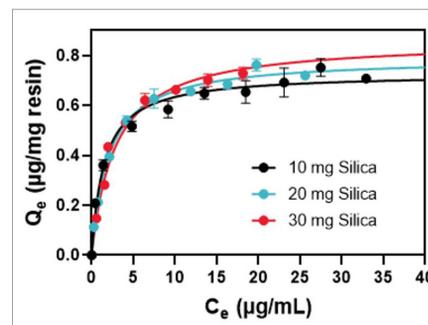


Figure 6. Binding Capacity of Silica Resin for pDNA. The plot compares the binding capacity (Q_e , $\mu\text{g}/\text{mg}$ resin) of three different amounts of silica resin (10 mg, 20 mg, and 30 mg) for pCRS 156 (5534 bp) plasmid. The equilibrium concentration (C_e) of the plasmid in solution is plotted against the bound plasmid (Q_e) for all resin quantities. Data is fitted to a Langmuir isotherm model.

Table 2: Maximum binding capacity (Q_{max}) for pCRS 156 (5534 bp) plasmid at 30 binding cycles, using different amounts of silica resin.

Silica Resin Amount	Q_{max} @ 30 cycles (5k)
10 mg	0.73 \pm 0.03
20 mg	0.79 \pm 0.02
30 mg	0.86 \pm 0.08

The theoretical binding limit (Q_{max}) at 30 cycles for all resin amounts is approximately 0.80 $\mu\text{g}/\text{mg}$ resin.

CONCLUSIONS

- A fully automated plasmid purification workflow for the Hamilton ML STAR automated liquid handling system that negates the need for additional off-deck steps and maintains yield and quality on par with traditional methods.
- Our optimized method demonstrates higher recoveries and purity compared to magnetic bead kits and comparable yields and purity to manual spin plates.
- Capable of efficiently processing up to 96 samples in less than 60 minutes, with high yields ($>10 \mu\text{g}$) and excellent purity.
- Binding capacity experiments indicate up to 24 μg of plasmid (5 kb) can be purified using 30 mg of silica.