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

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

Integrated Micro-Chromatography Systems, Inc., Irmo, SC

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.

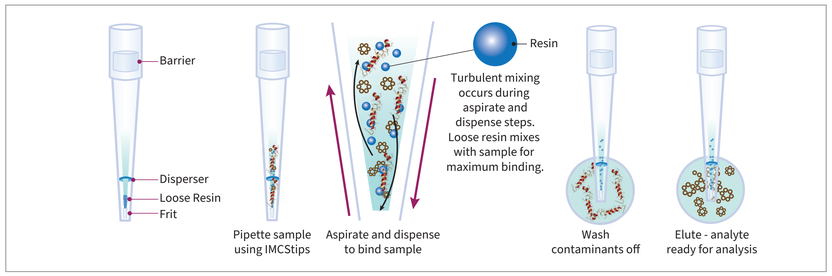

Figure 1: *IMCStips® containing loose resin employ dSPE to perform efficient automated extractions. Silica resins was used in IMCStips® to purify pDNA from bacteria cell lysate.*

METHOD

- Four plasmids of different sizes: pCRS158 (8484 bp), pCRS156 (5534 bp), pCRS492 (4105 bp), and pCRS 240.4 (3262 bp) in DH5α 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.

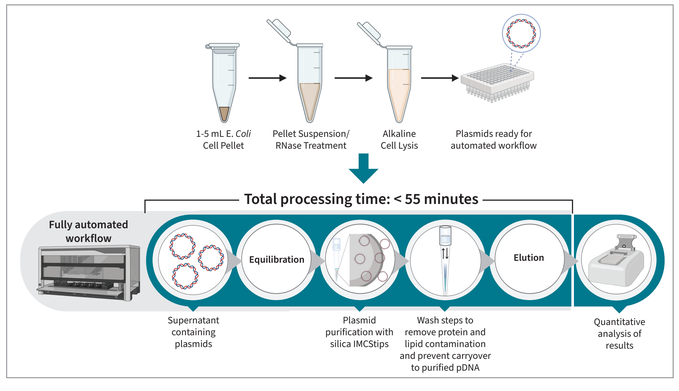

Figure 2. *Overview of the plasmid purification workflow on the Hamilton ML STAR.*

INSTRUMENTATION AND DECK LAYOUT

- Hamilton Microlab ML STAR.

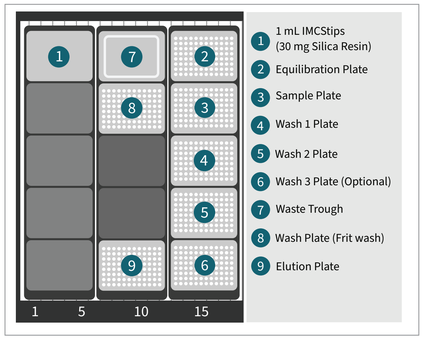

Figure 3. *Deck layout of the automated plasmid purification on a Hamilton ML STAR.*

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RESULTS

Comparing pDNA Yields and Purity Across Purification Kits

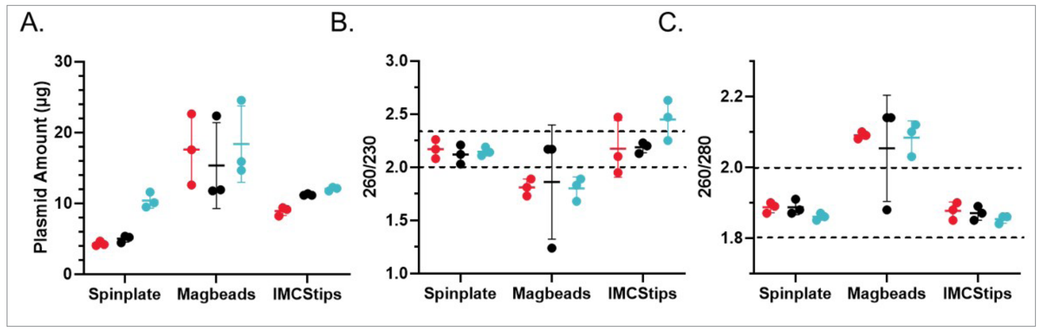

Figure 4. *Comparative analysis of plasmid DNA purification methods across different plasmid sizes.* ***A.)*** *Total yield of pDNA (µg) isolated using three different purification methods: spin plate, magbeads, and IMCStips®.* ***B.)*** *Purity of pDNA as indicated by the 260/230 nm absorbance ratio.* ***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 ± 1.70	4.29 ± 0.26	1.89 ± 0.0	2.17 ± 0.1	150
	5k	5534	33.37 ± 2.60	5.01 ± 0.39	1.89 ± 0.0	2.12 ± 0.0	150
	8k	8484	69.23 ± 5.90	10.39± 0.88	1.86 ± 0.0	2.15 ± 0.0	150
IMCStips (30 mg Silica Resin)	4k	4105	59.43 ± 3.30	8.92 ± 0.50	1.88 ± 0.0	2.17 ± 0.2	150
	5k	5534	74.73 ± 0.70	11.21 ± 0.10	1.87 ± 0.0	2.19 ± 0.0	150
	8k	8484	80.27 ± 1.40	12.04 ± 0.22	1.85 ± 0.0	2.45 ± 0.2	150
Magbeads	4k	4105	439.60 ± 102.3	17.58 ± 4.09	2.09 ± 0.0	1.81 ± 0.1	40
	5k	5534	383.37 ± 123.7	15.33 ± 4.95	2.05 ± 0.0	1.86 ± 0.4	40
	8k	8484	459.07 ± 110.1	18.36 ± 4.40	2.08 ± 0.0	1.80 ± 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®.

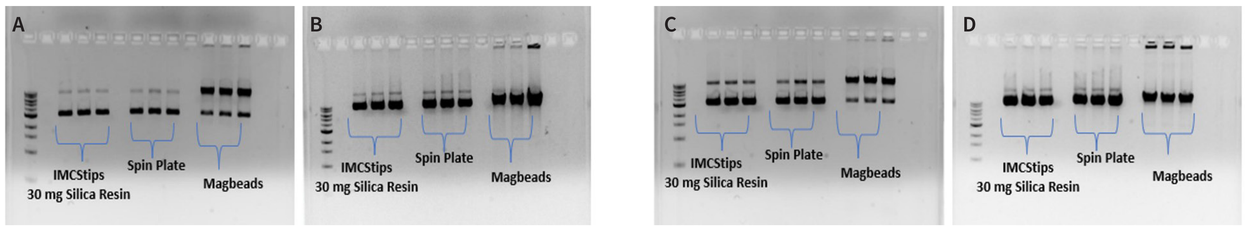

Figure 5. *Comparison of tip-purified (1 mL IMCStips® packed with silica resin) normalize spin plate/spinplate and mag bead and magbead.* ***Figures A and B*** *show 1 µL of eluted pDNA for the IMCStips, Spin Plate, and Magbeads methods for two plasmid sizes (4k, and 8k).* ***Figures C and D*** *show 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

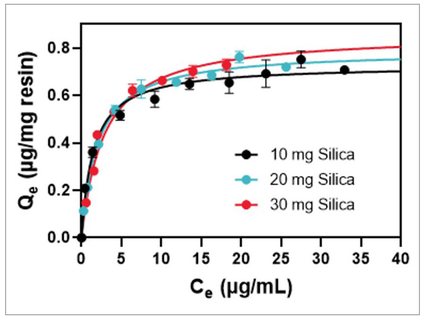

Figure 6. *Binding Capacity of Silica Resin for pDNA. The plot compares the binding capacity (Q_e, µg/mg resin) of three different amounts of silica resin (10 mg, 20 mg, and 30 mg) for pCRS 156 (5534 bp) plasmid. The equilibrium concentration (C_e) of the plasmid in solution is plotted against the bound 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 ± 0.03
20 mg	0.79 ± 0.02
30 mg	0.86 ± 0.08

The theoretical binding limit (Q_{max}) at 30 cycles for all resin amounts is approximately 0.80 µg/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 µg) and excellent purity.
- Binding capacity experiments indicate up to 24 µg of plasmid (5 kb) can be purified using 30 mg of silica.