Automated Plasmid Purification Workflow on the Dynamic Devices Lynx

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INTRODUCTION

Gram-negative bacteria are common vessels to produce plasmid DNA (pDNA). Due to the lipopolysaccharides (LPS) on the gram-negative bacterial cell membrane, endotoxin contamination is a main concern when obtaining transfection grade pDNA. LPS released during cell lysis have been shown to reduce transfection efficiencies and cell viability in endotoxin sensitive mammalian cells.[1] To avoid this, phase-based LPS removal is provided by many commercially available kits for the low endotoxin purification of pDNA. For high throughput laboratories, this process can be time consuming with multiple manual interventions using vacuum manifolds or gravity flow or magnetic beads. A novel approach is demonstrated where dispersive solid phase extraction (dSPE) in microPure LE IMCStips is used to purify 96 samples in an hour **(Figure 1)**.

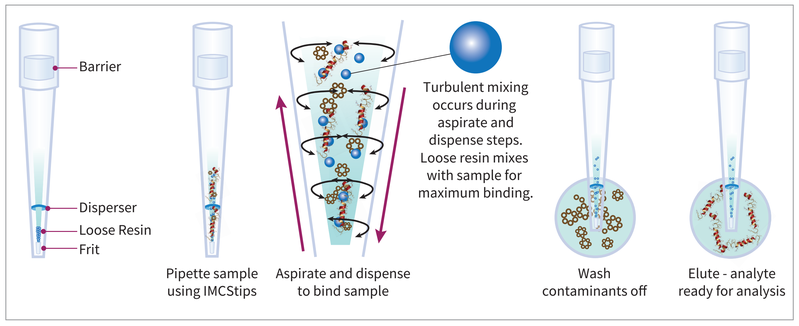
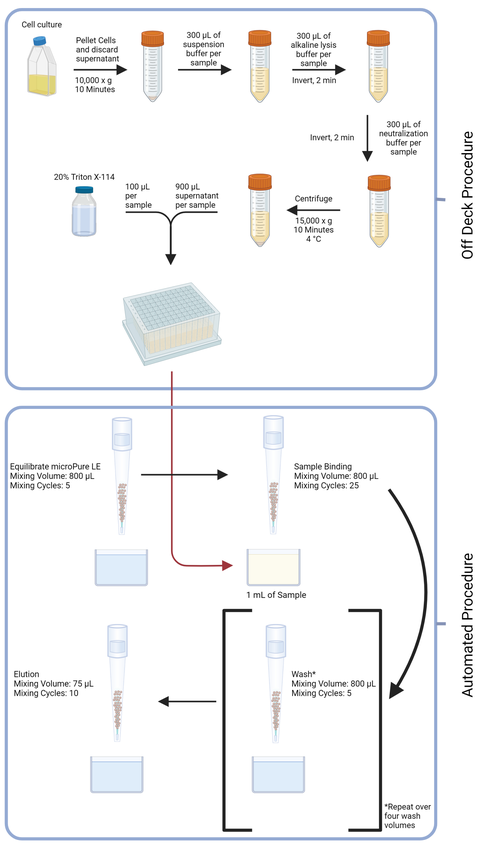


Figure 1. *microPure LE IMCStips containing loose resin employ patented, tip-based dSPE to perform efficient automated extractions of plasmid from cell lysate samples.*

METHODS

NEB 5α were transformed using three differently sized plasmids pCRS158 (8484 bp), pCRS166 (6258 bp), and pCRS240.3 (3593 bp) and stored in glycerol at -70 °C. The cells were cultured overnight (16-18 hrs) in Plasmid+ media (Thomson Instrument Co., Oceanside, CA, USA) containing 0.05 mg kanamycin per mL of liquid media prior to each experiment. The cells were pelleted and then resuspended in 300 µL of 50 mM Tris, 10 mM EDTA, pH 8.1 and 100 µg/mL RNase. The cells were lysed using an equivalent volume of 200 mM NaOH, 1% SDS lysis buffer, mixing by inversion for 2 minutes. The mixture was neutralized with 300 µL of 3 M potassium acetate, pH 5.5, then mixed by inversion for 2 minutes. After centrifugation at 15,000 x g for 10 minutes at 4 °C, 20% Triton X-114 was combined with the supernatant to a final concentration of 2% (v/v) and the mixture was transferred to a 96 well plate for processing **(Scheme 1)**.

For automated sample processing, the microPure LE IMCStips (P/N 04T-H6R33-1-25-96 IMCS, Irmo, SC) were used on the Lynx (LM1200), an automated liquid handling system from Dynamic Devices. The tips were equilibrated with equilibration buffer. Following plasmid binding, the tips were washed with 4 separate volumes of wash buffer. The pDNA was eluted using elution buffer **(Scheme 1)**. Purified samples were buffer exchanged into 1x TE buffer using SizeX IMCStips for downstream analysis. pDNA recovery was measured using a NanoDrop 2000. Pierce LAL Chromogenic Endotoxin Quantitation Kit (Thermo Scientific, Waltham, MA) was used according to the vendor's protocol.



Scheme 1. *The full off-deck and on-deck procedures for low endotoxin plasmid purification using microPure LE.*

RESULTS

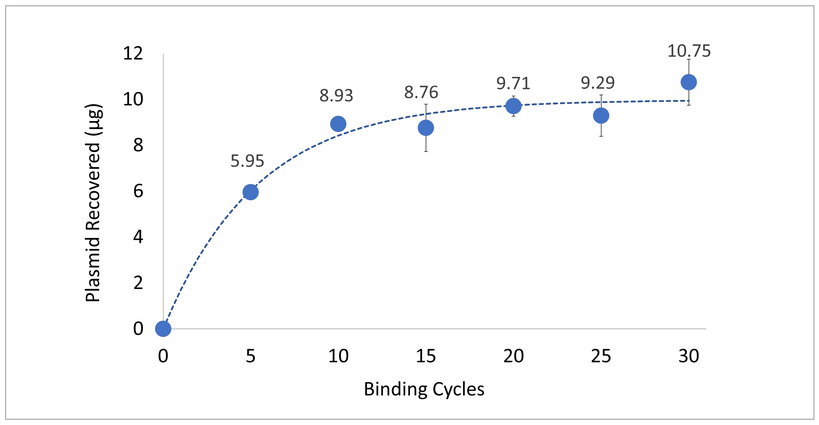


Figure 2. *Recovery of pCRS158 plasmid (8484 bp) from bacteria grown in Plasmid+ media. The yield of plasmid DNA capped at 10 binding cycles. (N = 3 IMCStips per binding cycle).*

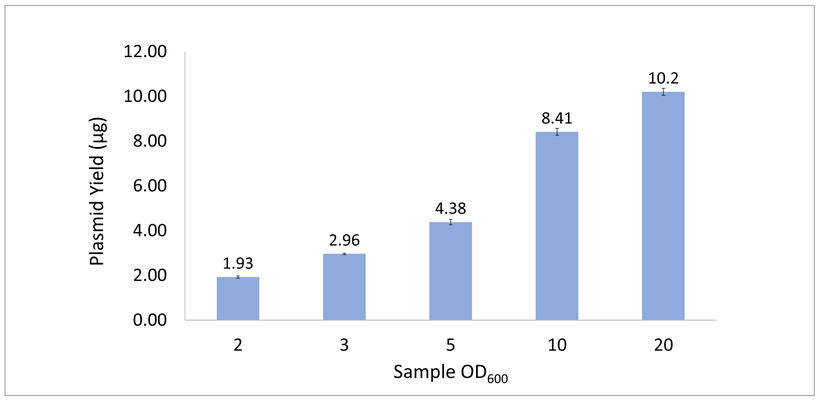


Figure 3. *Plasmid yields from bacteria cultured in Plasmid+ media. Varying cell densities represented by OD_{600} absorbance values were used (N = 3 per OD_{600}). Typical yields match OD_{600} levels, 2 OD yield was 2 µg, 3 OD being 3 µg, and starts to drop starting at 5 OD to 4.4 µg and 10 OD yield being 8.4 µg. 20 OD culture yield was 25% higher than 10 OD culture, either suggesting capacity is reached between 10-20 OD or cells quality is compromised. A fresh batch culture yielded close to 20 µg of plasmid, suggesting latter may be the case.*

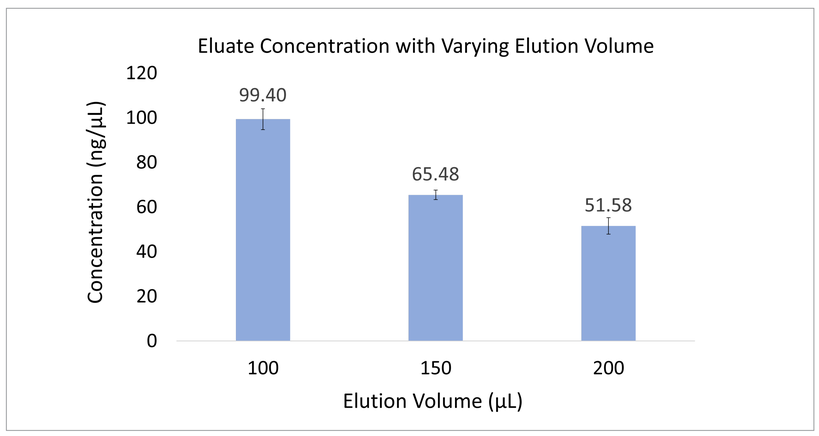


Figure 4. *Concentration of pCRS158 plasmid (8484 bp) in eluate. Bacteria were grown in Plasmid+ media to 20 OD_{600} (N = 4 per elution volume).*

Table 1. Comparison of microPure LE and Endofree Plasmid Maxi Kit (Qiagen) yield and absorbance ratios. Yield was normalized by volume of culture used for each sample for comparison. Samples were pCRS158 plasmid grown in Plasmid+ media to 15 OD_{600} (N = 7 for tips, N = 1 for kit).

Method	*Yield per mL Culture (µg)*	*260/280*	*260/230*
Tip	4.3 ± 0.5	1.87 ± 0.01	2.19 ± 0.04
Kit	4.7	1.90	2.28

Yield was normalized by volume of culture used for each sample for comparison. Samples were NEB5α containing pCRS158 plasmid, and these cells were grown in Plasmid+ media to 15 OD_{600} (N = 7 for tips, N = 1 for kit).

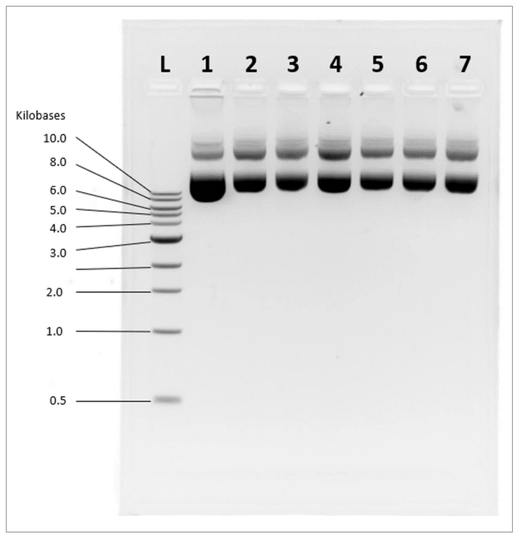


Figure 5. *The EndoFree™ Plasmid Maxi Kit prepared plasmid is in lane 1. 6 samples using microPure LE are in lanes 2-7 (lower concentrations due to post-run buffer exchange).*

Table 2. Yield and endotoxin concentration of pCRS166 and pCRS240.3. (N = 4 per plasmid).

Plasmid	*pCRS166*	*pCRS240.3*
Size (bp)	6258	3593
Sample OD_{600}	17	15
Yield (µg)	3.9 ± 0.9	5.0 ± 0.9
Endotoxin Content (EU/µg)	0.8 ± 0.7	0.5 ± 0.6

CONCLUSION

- Fully automated low endotoxin plasmid purification workflow for the Dynamic Devices Lynx automated liquid handling system.
- Able to purify over 10 µg of plasmid with eluate concentrations close to 100 ng/µL
- microPure LE IMCStips offer a convenient and time-efficient method to purify 96 individual low endotoxin preparations simultaneously.

CONFLICT OF INTEREST

The project is fully funded by Integrated Micro-Chromatography Systems, Inc (IMCS). L. Andrew Lee is an equity holder of IMCS.

REFERENCES

1. Butash, K. A.; Natarajan, P.; Young, A.; Fox, D. K. Reexamination of the Effect of Endotoxin on Cell Proliferation and Transfection Efficiency. BioTechniques 2000, 29 (3), 610–619.

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