Automated Workflow for Purification and Buffer Exchange of Low-Endotoxin Plasmid DNA

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INTRODUCTION

- Plasmid technology is a cornerstone in biotherapeutic applications
- High endotoxin content in purified plasmid samples can negatively impact downstream applications
- High throughput low-endotoxin plasmid purification is enabled via IMCStip technology
- Multi-modal programming on Hamilton STAR reduces off-deck handling
- ready-to-use product concentrated into a user-defined solution in under 1 hour

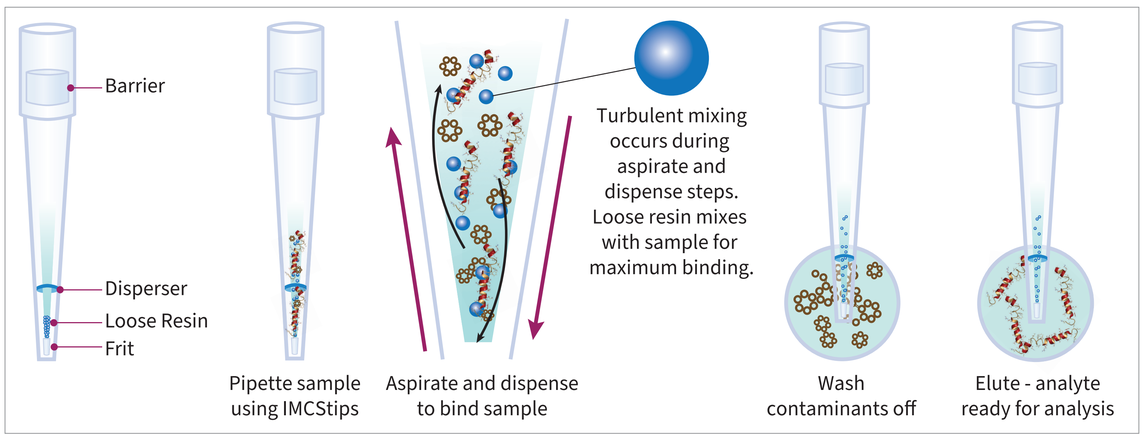

Figure 1. *IMCStips® containing loose resin employ INtip dSPE to perform efficient automated extractions.*

INSTRUMENTATION

- MicroPure LE IMCStips purify pDNA via anion-exchange
- INtip bidirectional flow allows for dynamic binding of plasmid
- pDNA eluates are top-loaded on to SizeX$_{100}$ tips
- Eluates are "chased" through the sizeX$_{100}$ resin bed to obtain buffer-exchanged product

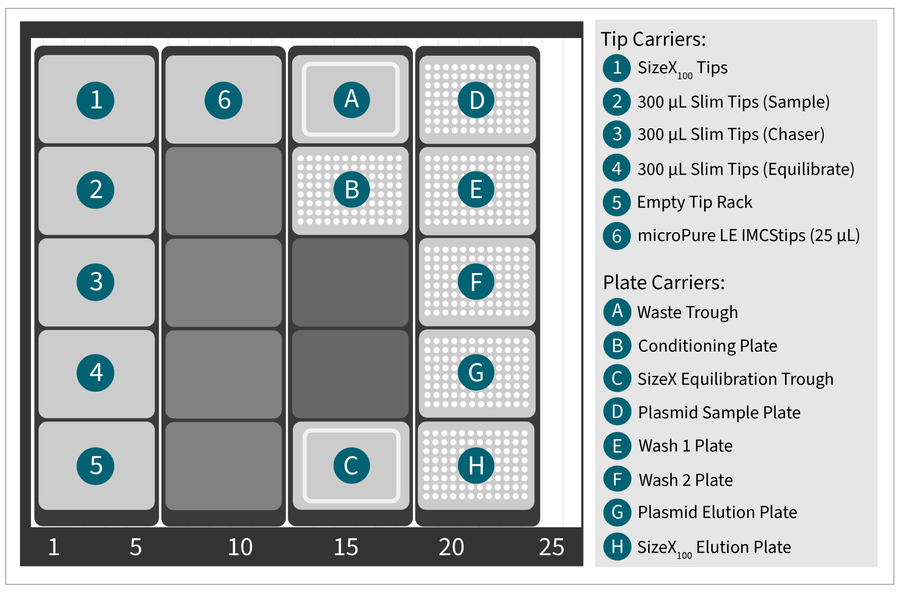

Figure 2. *Deck layout for the purification and buffer exchange of pDNA on the Hamilton Star.*

METHODOLOGY

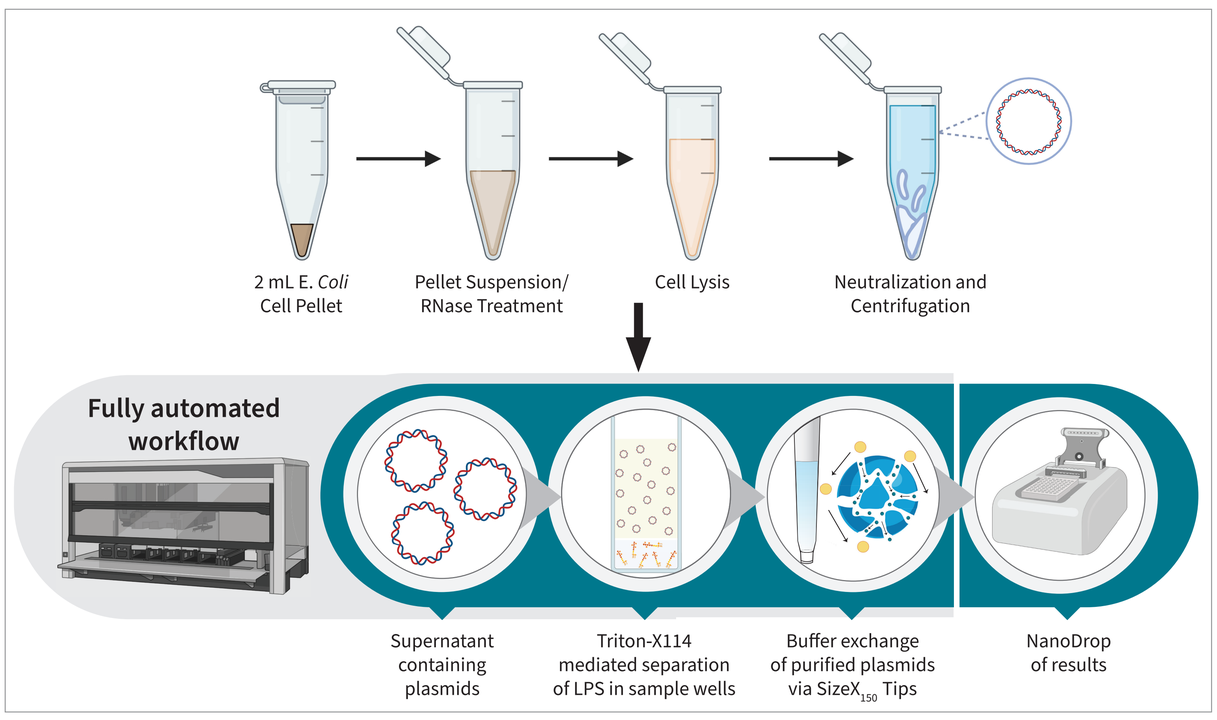

Figure 3. *Workflow for the preparation of pDNA samples and subsequent automated purification on the Hamilton STAR.*

- pCRS158 (8484 bp), pCRS166 (6258 bp), and pCRS240.3 (3593 bp) were transfected into NEB5α competent E.*coli* cells.
- Plasmid extraction via alkaline lysis
- 25 µL of anion exchange resin in 1 mL Hamilton tip for anion-exchange of pDNA
- 900 µL of lysate and 100 µL of 20% Triton-X114 per sample (n=8)
- Purified plasmid is buffer exchanged out of high salt and ethanol elution into a clean, concentrated solution of PBS.

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RESULTS

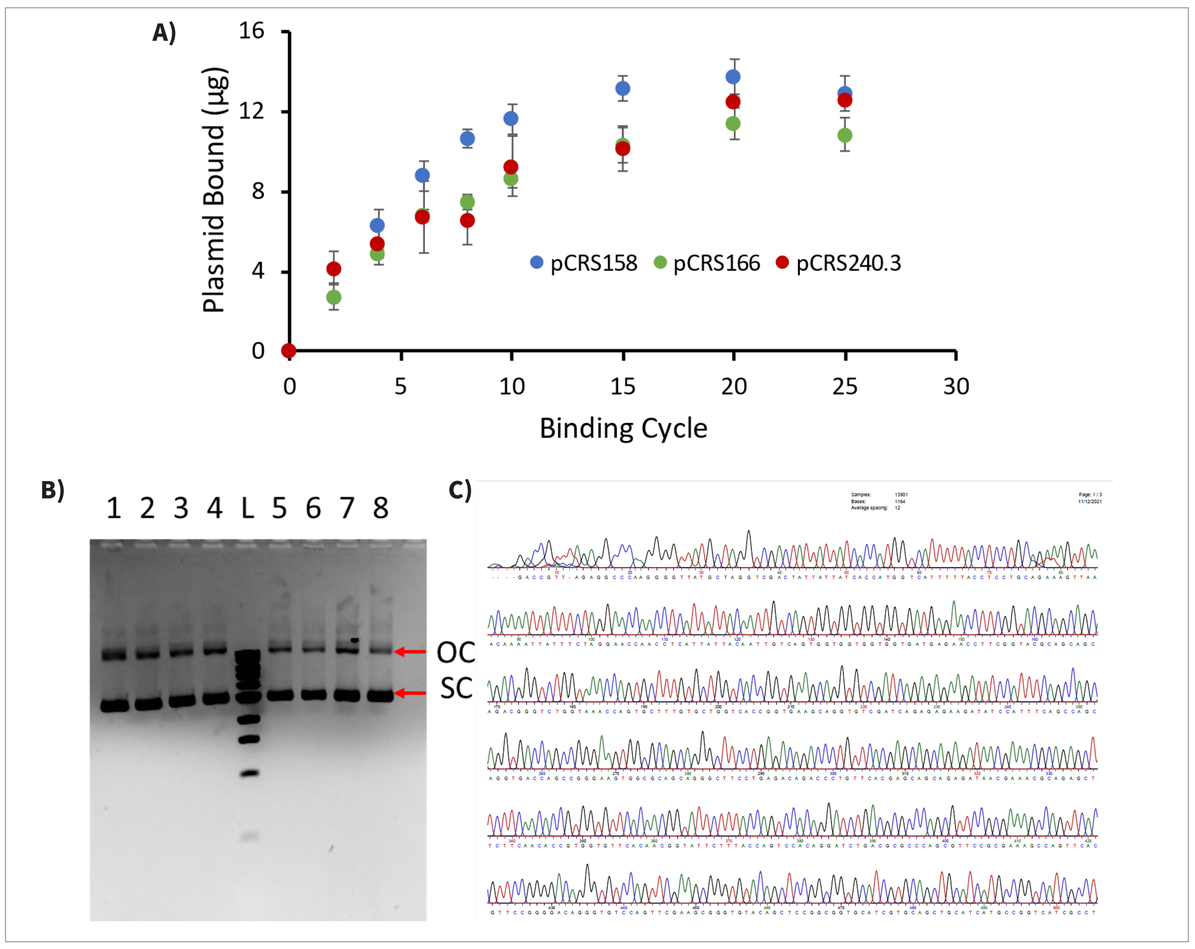

Figure 4. A) *Optimization of plasmid binding to microPure LE reveals that binding occurs exponentially with a plateau around 20th aspiration and dispense cycles.* ***B)*** *1.0 % agarose gel loaded with 50 ng of post SizeX$_{100}$ eluates for pCRS240.3 replicate samples. Supercoiled DNA (SC) runs further through the gel than open circle DNA (OC).* ***C)*** *Sequencing results for post SizeX$_{100}$ pCRS166 (76 ng/µL) demonstrate reliable downstream application.*

PLASMID RECOVERY PROFILE

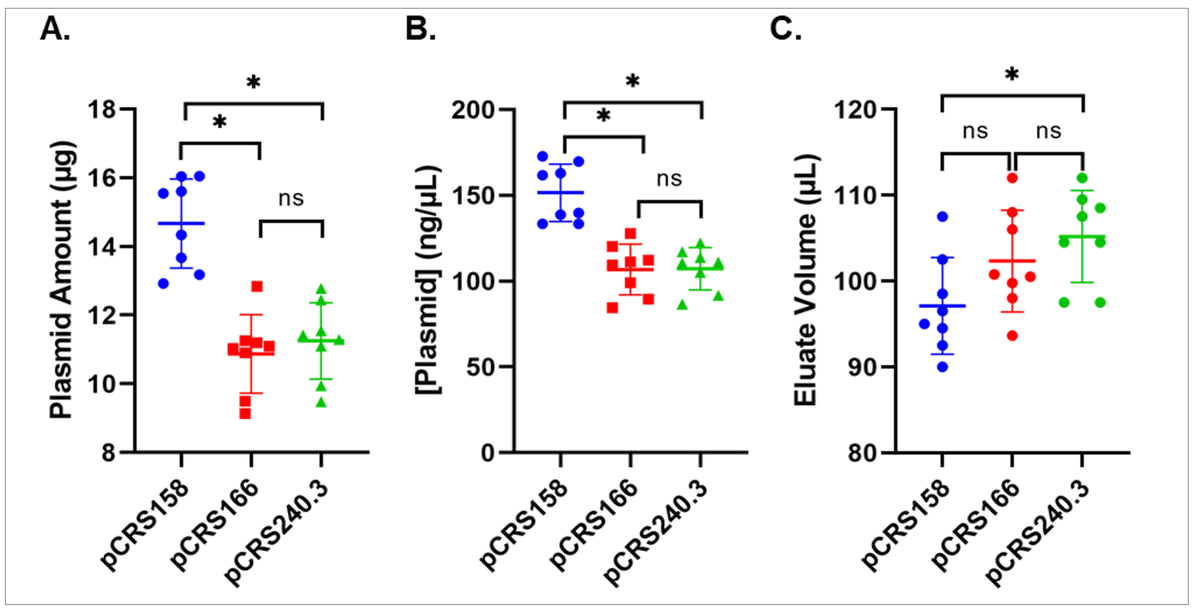

Figure 5. *The largest plasmid construct, pCRS158, demonstrated a significantly higher µg amount than pCRS166 and pCRS240.3 (* denotes p-value < 0.05)*

Table 1. TritonX-114 untreated samples (pCRS166) show ~34x higher endotoxin content than TritonX-114 treated samples.

	pCRS158	pCRS166	pCRS240.3
Plasmid size (bp)	8484	6258	3593
Total OD600	53.0	35.6	55.0
Post-SizeX Yield (µg)	14.6 ± 1.3	10.9 ± 1.1	11.3 ± 1.1
A260/280	1.88 ± 0.00	1.89 ± 0.03	1.89 ± 0.01
A260/230	2.27 ± 0.01	2.05 ± 0.07	2.37 ± 0.01
Plasmid Purified (pmoles)	47 ± 4.2	48 ± 4.8	87 ± 8.5
[Endotoxin] (EU/µg plasmid)	0.116 ± 0.110	3.36 ± 0.901	0.065 ± 0.109

CONCLUSIONS

- Our method works optimally when cultures are grown in Plasmid+ media, extracted with 20 sample binding cycles, and washed twice with equilibration/wash buffer.
- Our method provides >10 µg of desalted pDNA from 2 mL of overnight culture.
- Reproducible results of clean, buffer-exchanged pDNA product.
- 96 samples can be processed at one time and < 1 EU/µg per sample.
- Ready-to-use pDNA achieved in 57 minutes.

REFERENCES

1. Kates PA, Cook JN, Ghan R, Nguyen HJ, Sitasuwan P, Lee LA. Incorporation of automated buffer exchange empowers high-throughput protein and plasmid purification for downstream uses. *SLAS Technol.* Under Review. (2022).
2. Sousa Â, Sousa F, Queiroz JA. Advances in chromatographic supports for pharmaceutical-grade plasmid DNA purification. *J. Sep. Sci.* 35(22), 3046–3058 (2012).