

Automated Plasmid Minipreps with the Opentrons Flex[®] Automated Vacuum Module



Written by

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ABSTRACT

Plasmid DNA miniprep purification is a routine but labor-intensive step in molecular biology workflows, typically relying on manual vacuum manifolds or centrifugation to process silica-membrane spin columns in tubes or plates. As sample throughput increases, manual processing becomes a bottleneck and a source of errors and run-to-run variability. The Opentrons Flex paired with the Flex Automated Vacuum Module enables full automation of the miniprep workflow starting with *E. coli* cell pellets and finishing with eluted plasmid DNA without operator intervention. This approach reduces hands-on time, ensures user-to-user consistency, and scales cleanly from small validation runs to high-throughput plasmid preparation. Minipreps performed on the Automated Vacuum Module-enabled Flex are well suited for labs performing small-scale plasmid preps for PCR, cloning, sequencing, and/or in vitro transcription pipelines where consistent DNA quality is critical.

Key findings:

- **Walkaway time:** The automated protocol runtime for a single 96-well plate is ~55 minutes and is fully walkaway after 10 minutes of set up; partial plates can also be processed by sealing unused wells on the filter plates.
- **Plasmid yield:** From 1.5 mL of culture with a high-copy plasmid, the median concentration per sample was 91 ng/μL, with a total yield of 4.55 μg in an eluate volume of 50 μL.
- **Plasmid purity:** Plasmid purity was high with little-to-no contamination of protein, organic compounds or salts as evidenced by an average A260/A280 of 1.83 ± 0.01 and an average A260/A230 of 2.12 ± 0.06 .
- **Cross-contamination:** Cross-contamination between wells was undetectable via a 25-cycle PCR reaction comparing eluate from sample wells versus adjacent empty wells as template.
- **Downstream applications:** Purified plasmid DNA successfully served as input for standard downstream applications including restriction digestion, PCR, and Sanger sequencing reactions.
- **Per-sample cost:** At ~\$2.16 per sample, inclusive of pre-made buffers, the cost is on par or up to 2-fold lower in cost than commercially available 96-well miniprep kits and in range with single-column kits.

INTRODUCTION

Current approaches: Plasmid DNA purification is one of the most frequently performed procedures in molecular biology, underpinning workflows such as cloning, PCR, sequencing, and in vitro transcription. Silica-membrane spin columns have become the standard format for plasmid minipreps due to their speed and simplicity. Processing these columns typically relies on manual vacuum manifolds or benchtop centrifugation to drive lysate and wash buffers through the membrane.

Drawbacks of current approaches: As sample number increases up to full 96-well plates, manual processing becomes a significant bottleneck. Each step, from resuspension, lysis, and neutralization, to clarification, binding, washing, and elution, requires continuous operator pipetting and plate handling. Beyond the time cost, manual workflows are a common source of error and run-to-run variability; pipetting inconsistencies, timing differences between operators, and the physical strain of repetitive vacuum or centrifugation steps can all affect DNA yield, purity, and reproducibility, particularly in labs where multiple people run the same protocol.

Automated solution: The Opentrons Flex paired with the Flex Automated Vacuum Module addresses throughput bottlenecks and reproducibility issues by automating the entire miniprep workflow, from resuspension of *E. coli* pellets through to final elution, without operator intervention. This application note describes a Flex protocol designed to process a single 96-well sample plate in ~1 hour. Plasmid yield and purity, well-to-well cross-contamination, and compatibility with standard downstream applications are evaluated, demonstrating that automation can match or exceed manual methods in quality while eliminating hands-on time and user-to-user variability.

RESULTS

Automated processing completes a 96-well plate in under an hour with only 10 minutes of hands-on set up time.

The automated miniprep method described here runs on the Opentrons Flex and begins with on-deck alkaline lysis of *E. coli* pellets: resuspension in P1 buffer, lysis with P2 buffer, and neutralization with P3 buffer. Plasmid DNA is then purified using the Flex Automated Vacuum Module, which clarifies the lysate and performs binding, washing, and elution into a fresh collection plate (**Figure 1**). A single 96-well plate is processed in 54 minutes: 10 minutes of setup time, during which overnight cultures are pelleted by centrifugation, followed by 44 minutes of fully walkaway processing. An equivalent manual protocol using a vacuum manifold with house air or a pump takes a comparable 57 minutes but requires 100% hands-on time throughout and, as with any manual process, is prone to user error and run-to-run variability.

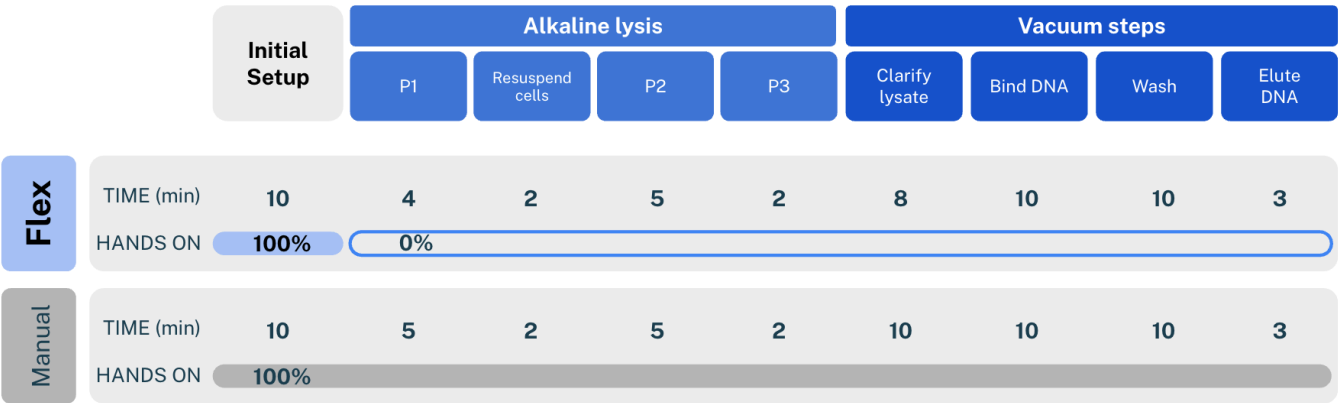


Figure 1. Automated miniprep on the Flex versus manual.

The automated protocol delivers consistent, high-yield, high-purity plasmid DNA across a full 96-well plate.

Plasmid DNA purified using the automated miniprep protocol on the Flex was quantified by NanoDrop spectrophotometry, with A260/280 and A260/230 ratios used to assess purity. For this experiment, 1.5 mL from a single 100 mL *E. coli* culture was distributed in a checkerboard pattern into 48 wells of a 96-deep-well plate, alternating with 48 empty wells (**Figure 2A**), to allow simultaneous assessment of yield, purity, and well-to-well cross-contamination. Plasmid concentration was consistent across the 48 sample wells, with a median of 91 ng/μL and a tight quartile range of 84 - 98 ng/μL (**Figure 2B**), indicating uniform processing across the plate. At an elution volume of 50 μL, this corresponds to a median yield of 4.55 μg of plasmid DNA per well. Purity was high, with little to no contamination by protein, organic compounds, or salts, as shown by an average A260/280 of 1.83 ± 0.01 and an average A260/230 of 2.12 ± 0.06. Eluate from four empty wells showed only background signal on the NanoDrop.

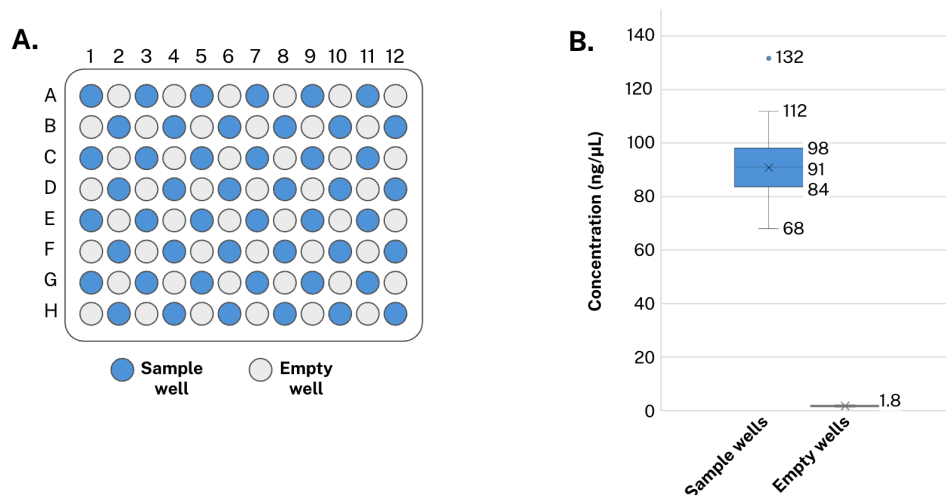


Figure 2. Sample plate layout (A) and plasmid concentration by well type (B).

Vacuum-based purification produces no detectable cross-contamination between wells. Vacuum-based nucleic acid purification can be prone to cross-contamination, as filtrate exiting the filter plate may enter neighboring wells during vacuum application. To mitigate this risk, the Flex protocol specifies stacking the DNA-binding plate directly on top of the collection plate within the collar in order to eliminate the vertical gap between the two plates. To test for cross-contamination, eluate from wells in column 7 was diluted 1:100 and subjected to PCR using primers expected to produce a ~1 kb amplicon. Following 25 PCR cycles, 10% of the reaction volume was run on a 1% agarose gel (**Figure 3**). All four sample wells (A7, C7, E7, G7) showed strong amplicons at the expected size, while empty wells (B7, D7, F7, H7) showed no amplification. Together, these results indicate that cross-contamination between wells resulting from vacuum steps is negligible.

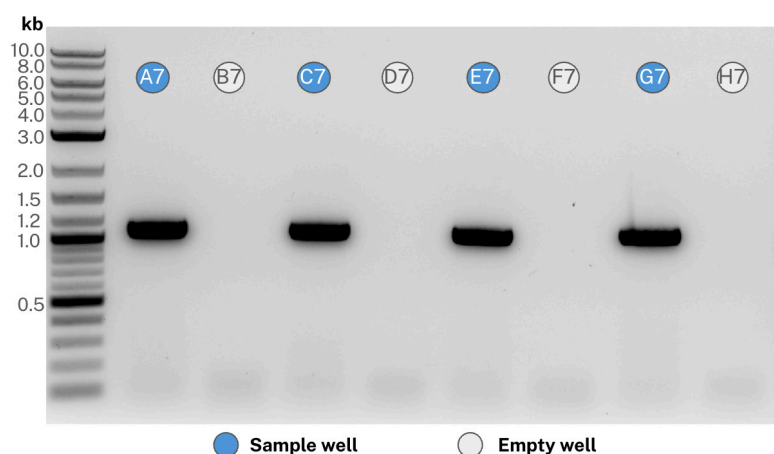


Figure 3. Cross-contamination assessment by PCR and gel electrophoresis of eluate from column 7.

Plasmid DNA from the automated protocol performs reliably in restriction digestion, PCR, and Sanger sequencing. Common downstream applications for miniprep plasmids include PCR, restriction digestion, and Sanger sequencing. Plasmids from sample wells performed well as template for PCR, as evidenced in the cross-contamination test (**Figure 3**). To evaluate plasmids purified with the automated protocol for other applications,

eluate from eight sample wells was subjected to double restriction digestion or Sanger sequencing. On a 1% agarose gel, uncut plasmid samples behaved as expected, showing multiple plasmid species (supercoiled, linear, and nicked), indicating high-quality plasmid preparations (**Figure 4A, Uncut**). Following double digestion, all eight samples produced the expected two bands (**Figure 4A, Cut vs Figure 4B**). All eight samples also yielded high-quality Sanger sequencing results, with quality scores generally exceeding 40 and read length exceeding 800 base pairs (**Figure 4C**). Together, these results demonstrate that plasmids purified using the automated miniprep protocol on the Flex are of high quality and are suitable substrate for standard downstream applications.

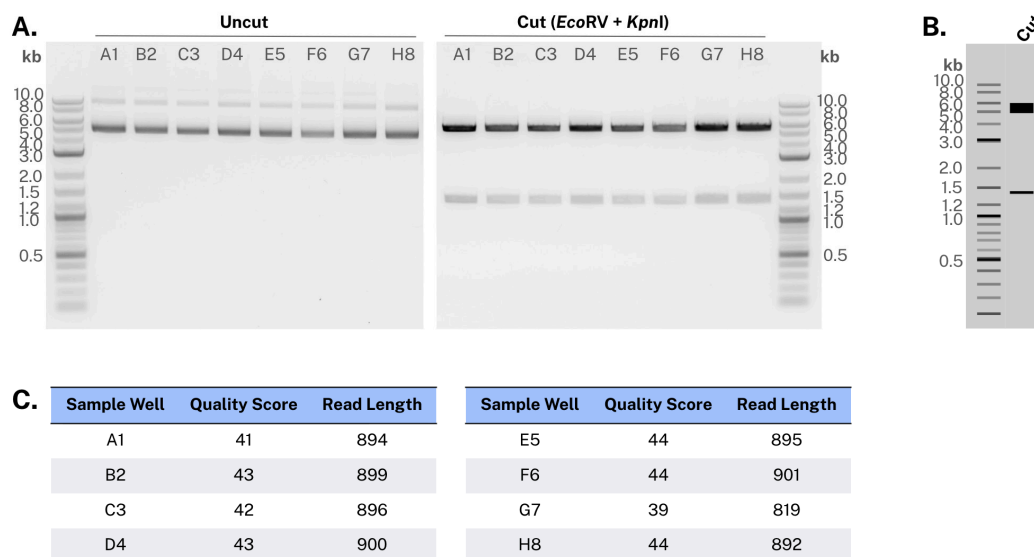


Figure 4. Plasmid performance in restriction digest (A) compared to expected pattern B) and Sanger sequencing (C).

Automation matches or beats commercial kit pricing while returning ~45 minutes of hands-on time per plate.

Commercial 96-well plasmid purification kits are expected to be compatible with automation on the Flex Automated Vacuum Module. The automated protocol presented here costs \$2.16 per sample in reagents and consumables (Table 1), on par with, or up to ~2-fold lower than, common 96-well miniprep kits, and further reducible by preparing buffers in-house rather than purchasing them pre-made. Since unused filter-plate columns can be sealed off to process partial plates, we also benchmarked per-sample cost against three common single-column kits; costs came out roughly comparable across all three, though the automated protocol still eliminates the hands-on pipetting error inherent to single-tube processing.

Table 1. Per-sample cost of the automated protocol compared to commercial 96-well and single-column miniprep kits.

Format	Source	Per-sample cost*
96-well plate	Opentrons automated protocol	\$2.16
96-well plate	Commercial kit 1	\$2.39 - 2.92
96-well plate	Commercial kit 2	\$2.41 - \$3.21
96-well plate	Commercial kit 3	\$4.10
Single-column	Commercial kit 1	\$1.58 - \$3.90
Single-column	Commercial kit 2	\$2.03 - \$2.56
Single-column	Commercial kit 3	\$1.47 - \$2.34

* range depends on kit size purchased

Automation pays for itself in under a year. Beyond reagent cost, the automated protocol returns approximately 45 minutes of hands-on operator time per 96-well plate relative to the equivalent manual protocol. At a throughput of one plate per day, this corresponds to roughly 15 hours of operator time recovered per month, valued at approximately \$2,250 per month assuming a fully loaded operator cost of \$150/hour. Against the \$15,000 cost of the Flex Automated Vacuum Module for labs already equipped with a Flex, this time savings alone recovers the hardware investment in approximately 7 months, with the payback period shortening further as plate throughput increases.

CONCLUSION

The Opentrons Flex, equipped with the Flex Automated Vacuum Module, enables fully automated plasmid miniprep purification directly from *E. coli* pellets to eluted DNA, with no operator intervention required after setup. A single 96-well plate is processed in under an hour, with only 10 minutes of hands-on setup time, compared to a similar total runtime but 100% hands-on time for the equivalent manual vacuum manifold protocol. This shift from manual to walkaway processing removes a major bottleneck in labs running plasmid preps at scale, while also eliminating the operator-to-operator variability inherent to manual pipetting and handling. While this protocol specifies processing a single 96-well plate, the addition of Stackers to the Flex configuration and revisions to the protocol can increase the number of plates processed in a single run to 4 or more.

Beyond throughput, the automated protocol produces plasmid DNA of consistently high yield and purity, with negligible well-to-well cross-contamination even in a dense 96-well format. Plasmids purified this way performed reliably across standard downstream applications, including restriction digestion and Sanger sequencing, indicating that automation does not come at the cost of DNA quality.

Together, these results support the Flex Automated Vacuum Module as a robust solution for R&D labs seeking to scale plasmid miniprep purification, whether processing a handful of validation samples or full plates, without sacrificing consistency, purity, or compatibility with downstream molecular biology workflows.

METHODS

***E. coli* pre-culture and plasmid.** Top10 *E. coli* cells transformed with a 6.8 kb, high-copy, ampicillin resistant plasmid (Figure 5), were inoculated in 100 mL LB plus 100 µg/ml ampicillin and grown for ~20 hours at 37°C with orbital shaking at 225 rpm to an OD600 of 2.64. 1.5 mL of culture was distributed in a checkerboard pattern into 48 wells of a 96-deep well plate. Cell pellets were collected by centrifugation at 4000 g for 10 minutes at room temperature and supernatant was dumped off manually by flicking.

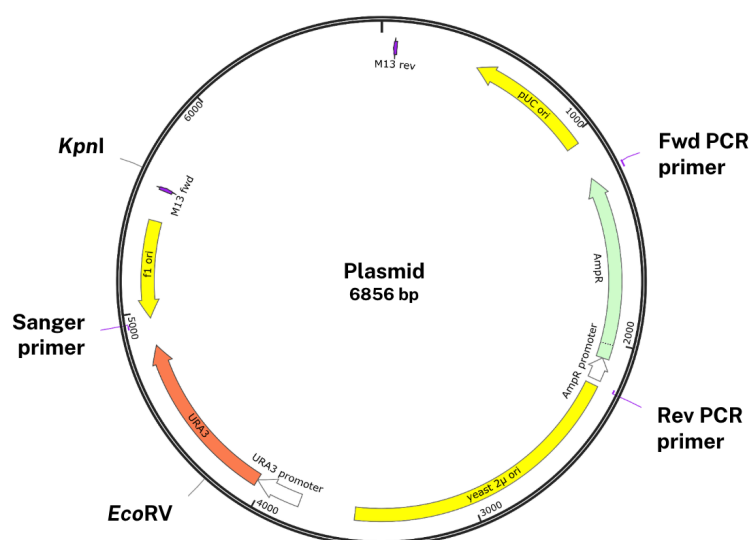


Figure 5. Plasmid map showing restriction enzyme and primer binding sites.

Flex configuration and deck layout. The configuration of the Flex Vacuum Purification Workstation includes a 96-channel head, a gripper, a waste chute, the Flex Automated Vacuum Module, with the heater-shaker module added for this protocol. The deck was laid out as shown in **Figure 6**.

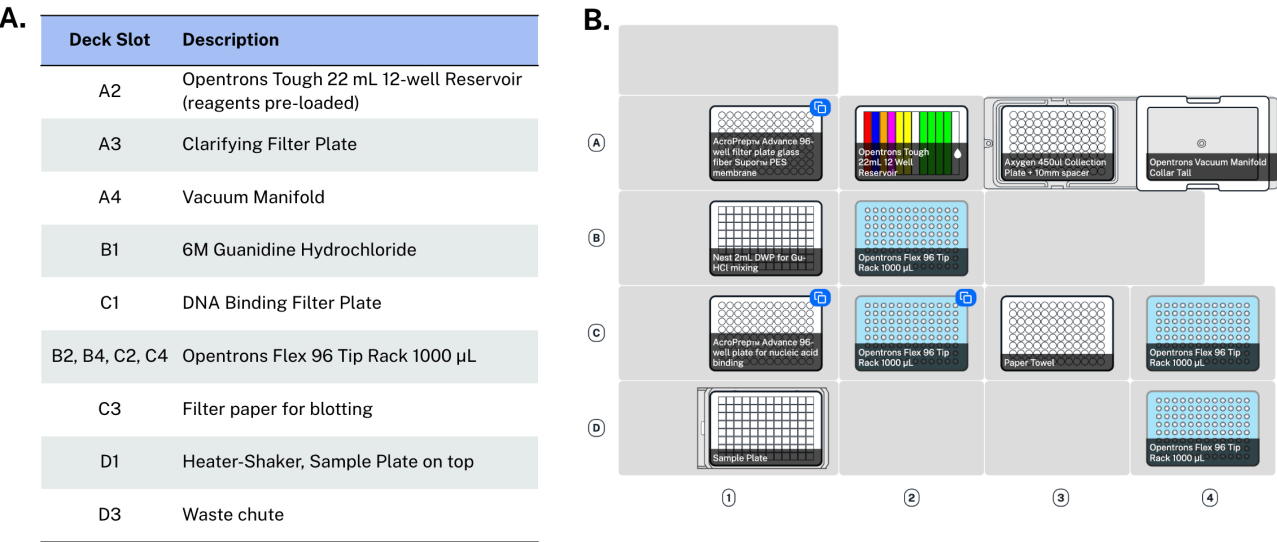


Figure 6. Flex deck layout.

Hardware, reagents and consumables. Hardware, reagents and consumables used in the Automated miniprep protocol are shown in **Table 2**, **Table 3**, and **Table 4**, respectively.

Table 2. Hardware used in the automated miniprep protocol.

Hardware	Vendor	Product Number
Flex	Opentrons	999-00191
Gripper	Opentrons	999-00194
96-channel pipette 5-1000 uL	Opentrons	999-00196
Vacuum Module	Opentrons	999-00296
Heater Shaker	Opentrons	991-00115-FL-UN
Waste Chute	Opentrons	999-00227

Table 3. Reagents used in the automated miniprep protocol.

Buffer	Composition	Manufacturer	Product Number	List Price	Volume
Resuspension Buffer (P1)	50 mM Tris-HCl pH 8.0, 10 mM EDTA, 100 ug/ml RNase A	Qiagen	19051	\$110	500 mL
Lysis Buffer (P2)	200 mM NaOH, 1% SDS	Qiagen	19052	\$109	500 mL
Neutralization Buffer (P3)	3.0 M Potassium Acetate, pH 5.5	Qiagen	19503	\$107	500 mL
Binding Buffer	6 M Guanidine-HCl	Abcam	ab289852	\$240	1000 mL
Wash Buffer	10 mM Tris-HCl pH 7.5, 80% Ethanol	Qiagen	19055	\$180	1000 mL
Elution Buffer	10 mM Tris-HCl pH 8.0, 1 mM EDTA	Qiagen	19056	\$186	1000 mL

Table 4. Consumables used in the automated miniprep protocol.

Consumable	Purpose	Manufacturer	Product Number	Price per unit	Cost per 96 samples
Flex Tips, 1000 uL filtered (20 refills)	Liquid transfers	Opentrons	991-00109	\$15.00	\$60.00
NEST 2 mL 96-Well Deep Well Plate (50/pk)	Sample plate; Guanidine HCl plate	Opentrons	999-00103	\$8.00	\$16.00
AcroPrep™ Advance 96-well filter plate, 3 µm glass fiber/0.2 µm Supor™ PES membrane, 1 mL (5/Pk)	Clarifying filter plate	Cytiva	8175	\$38.40	\$38.40
96-Well, V-Bottom 500 µL Polypropylene Deep Well Plate, Clear, Sterile	Collection plate	Axygen	P-96-450V-C-S	\$4.08	\$4.08
AcroPrep™ Advance 96-well filter plate, DNA binding glass fiber membrane, long tips, 1 mL (5/Pk)	DNA SPE filter plate	Cytiva	8133	\$43.80	\$43.80
0.2 mL 96-well PCR Plate Full Skirt (25/pk)	Elution plate	Opentrons	991-00076	\$9.80	\$9.80
Tough 22 mL 12 Well Reservoir (25/pk)	Buffers	Opentrons	999-00260	\$6.80	\$6.80
Buffers*	P1, P2, P3, etc.	various	various	various	\$28.80
Total cost per 96 samples					\$207.68
Total cost per 1 sample					\$2.16

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