Application Note

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INTEGRA

Automated ultrapure plasmid DNA preparation with the ZymoPURE™ 96 Plasmid Miniprep Kit

Introduction

Automated plasmid purification is a revolutionary technique that streamlines the isolation of transfection-grade plasmid DNA from bacterial cultures. It combines automated liquid handling with advanced purification methods to eliminate labor-intensive manual steps, reduce human errors and increase throughput. Traditionally, obtaining plasmid DNA pure enough for mammalian cell transfection was a major bottleneck due to slow gravity-flow columns, laborious precipitation steps and multiple rounds of culturing and verification, making the process poorly suited for high throughput workflows. Automation has transformed the field of molecular biology, allowing researchers to efficiently obtain large quantities of high quality plasmid DNA for various applications, such as cloning, gene expression studies and genetic engineering.

The ZymoPURE 96 Plasmid Miniprep Kit, developed by Zymo Research, uses a modified alkaline lysis method and novel high binding chemistry to streamline the purification of plasmids, resulting in up to 100 µg of ultrapure transfection-grade plasmid DNA. This process can be automated using a VIAFLO electronic pipette on an ASSIST PLUS pipetting robot equipped with an EZ-Vac 96 vacuum manifold, eliminating user errors and drastically reducing tedious liquid handling steps for high throughput applications. In this application note, we show the high binding capacity and ability to obtain ultrapure high yielding plasmid DNA when using the ZymoPURE 96 Plasmid Miniprep Kit on the automated ASSIST PLUS.

Key benefits:

- Reliable, high throughput processing of 96 samples with the ZymoPURE 96 Plasmid Miniprep Kit on the ASSIST PLUS pipetting robot using an on-deck vacuum manifold.
- Automated workflow eliminates errors and frees user from tedious and time-consuming liquid handling steps, while the VIAFLO electronic pipette guarantees accurate and precise liquid handling on the ASSIST PLUS.
- ZymoPURE's patented technology provides highly concentrated plasmid DNA suitable for transfection, transformation, CRISPR, sequencing and other sensitive downstream applications.
- Optimized wash regime with separate wash plates reduces cross-contamination and ensures ultrapure, transfection-grade plasmid DNA free from endotoxins, salts, proteins and RNA.

Overview: How to automate plasmid miniprep on the ASSIST PLUS with the ZymoPURE 96 kit

ASSIST PLUS

Application Note

This application note describes a protocol of plasmid purification of up to 5 ml of overnight *E. coli* JM109 (pGL3®) culture using the automated miniprep protocol on the ASSIST PLUS pipetting robot with the ZymoPURE 96 Plasmid Miniprep Kit and the EZ-Vac 96 vacuum manifold.

Experimental set-up

The ASSIST PLUS, together with the 8 channel 1,250 µl VIAFLO electronic pipette and 1,250 µl sterile, filter GRIPTIPS® pipette tips, automates all liquid handling steps in one program consisting of the following steps (**Figure1**):

- Lyse culture pellet and filter neutralized lysates
- Bind plasmid DNA
- Wash plasmid DNA
- Elute plasmids DNA

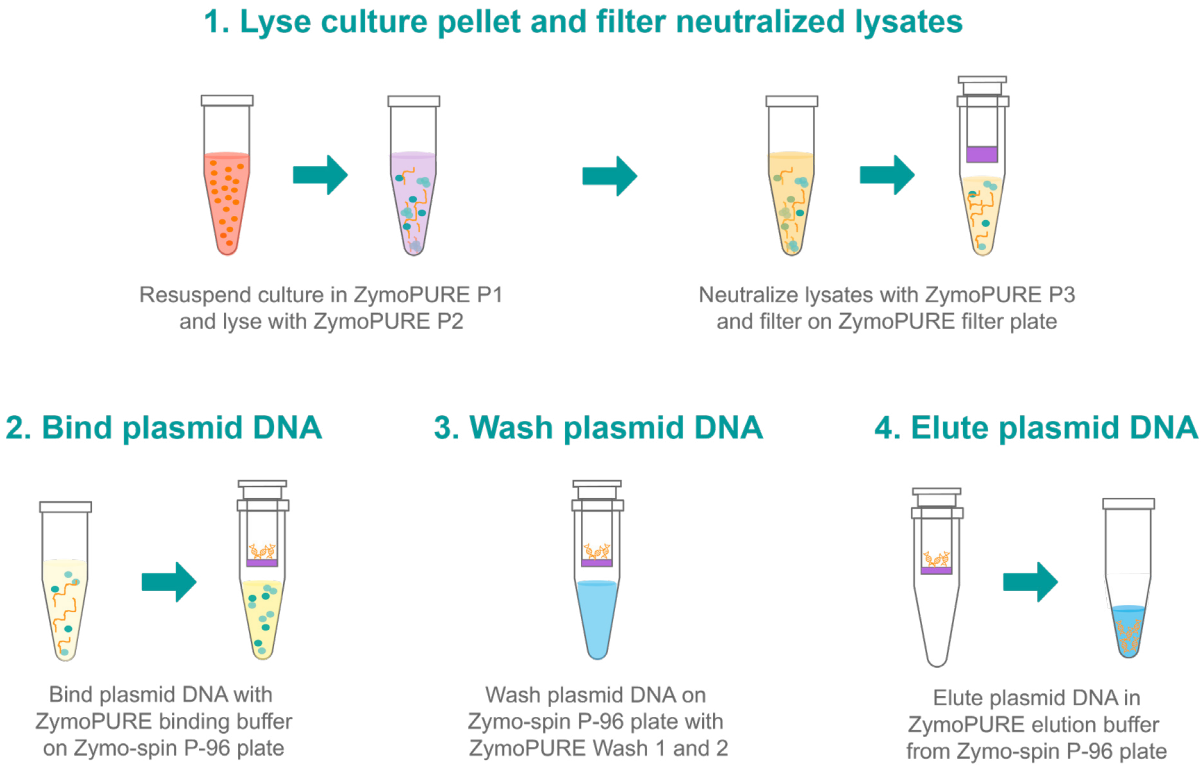


Figure 1: Steps of the automated miniprep protocol.

Step-by-step procedure

Step 1: **Lyse culture pellets and filter neutralized lysates**

HOW TO: Place an automation-friendly 8 row reservoir insert and base in portrait orientation on position A (**Figure 2**). Fill the whole bottle volume (at least 28 ml) of ZymoPURE P1 in column 1 (**Figure 2**, red), ZymoPURE P2 in column 2 (**Figure 2**, light blue), ZymoPURE P3 in column 3 (**Figure 2**, yellow) and ZymoPURE binding buffer in column 4 (**Figure 2**, light green). Place the deep well plate (DWP) containing *E. coli* pellets in each well in landscape orientation on position B (**Figure 2,** orange).

The EZ-Vac 96 vacuum manifold can be assembled with the provided labware from the ZymoPURE 96 Plasmid Miniprep Kit in 3 different ways:

- EZ-Vac 96 set-up A (**Figure 3**)
- EZ-Vac 96 set-up B (**Figure 6**)
- EZ-Vac 96 set-up C **(Figure 3**)

First, assemble the EZ-Vac 96 set-up A, as shown in **Figure 3a**. Put the manifold bed with the DWP in position C (**Figure 3a**) with the manifold collar that accommodates the ZymoPURE filter plate on top (**Figure 3b**). Connect the tubing to the vacuum pump.

Tips:

- Samples can be transferred between different labware formats. With the MINI 96 or VIAFLO 96 electronic pipettes, the *E. coli* culture can be transferred from an automation-friendly reservoir to the DWP in one step.
- Media transfer and inoculation can be sped up with the MINI 96 or VIAFLO 96.

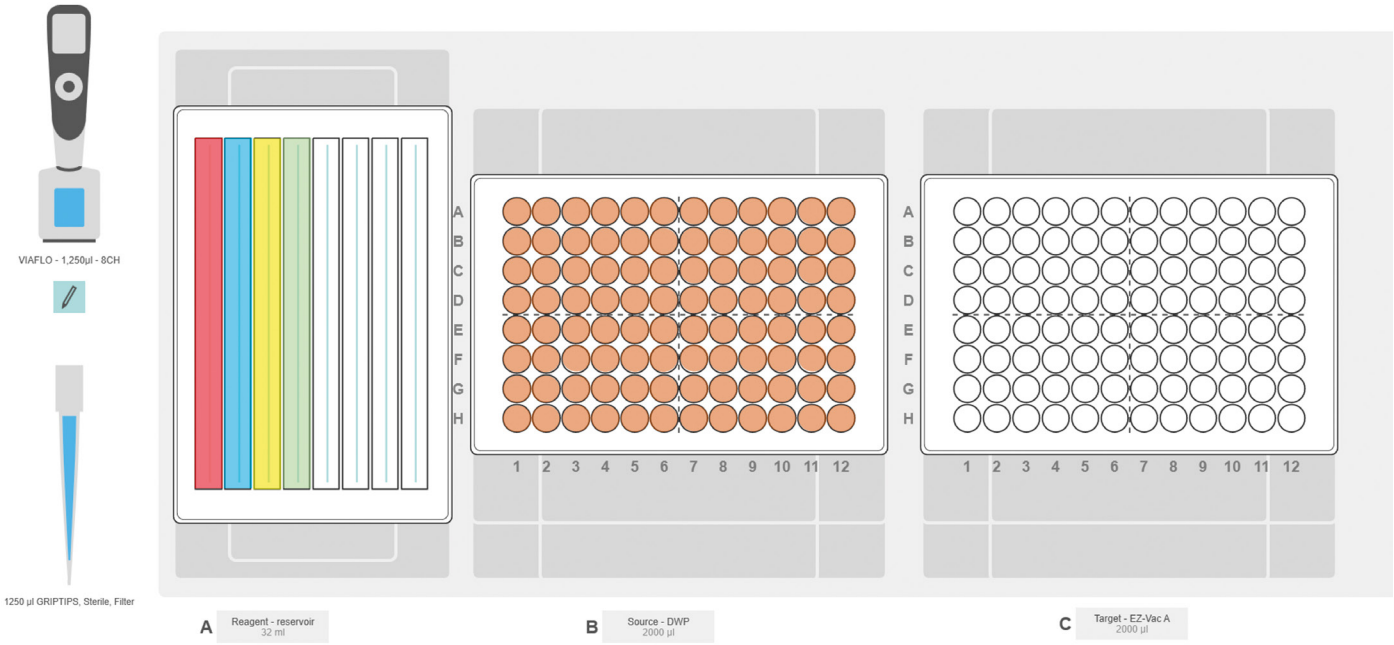


Figure 2: Deck set-up with the EZ-Vac 96 set-up A. **Position A**: Reagents – 8 row polypropylene reservoir insert with base (Red – ZymoPURE P1; Light blue – ZymoPURE P2; Yellow – ZymoPURE P3; Light green – ZymoPURE binding buffer) **Position B**: Source – Deep well plate (DWP) with *E. coli* pellets (orange). **Position C**: Target – EZ-Vac 96 set-up A.

a)

b)

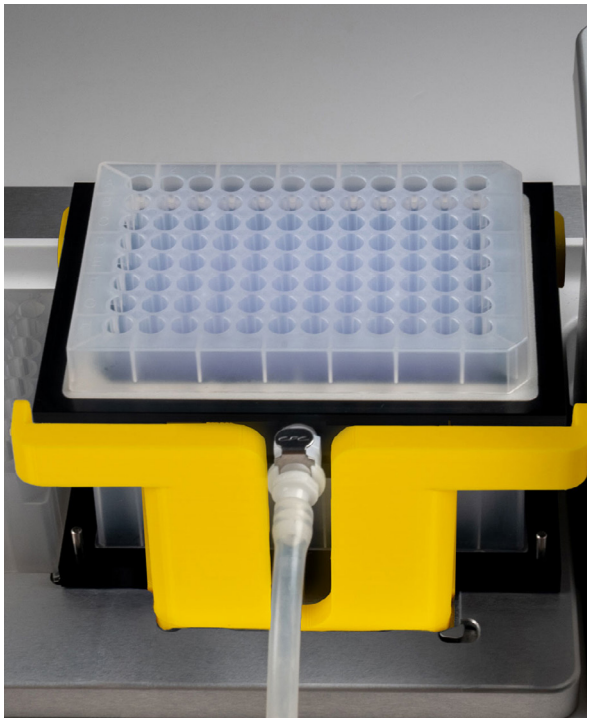


Figure 3: EZ-Vac 96 set-ups A and C on the ASSIST PLUS. a) The manifold bed and DWP are placed on deck position C. b) The manifold collar with the tubing connection is placed onto the DWP, and accommodates the ZymoPURE filter plate for set-up A and the Zymo-Spin P-96 plate for set-up C.

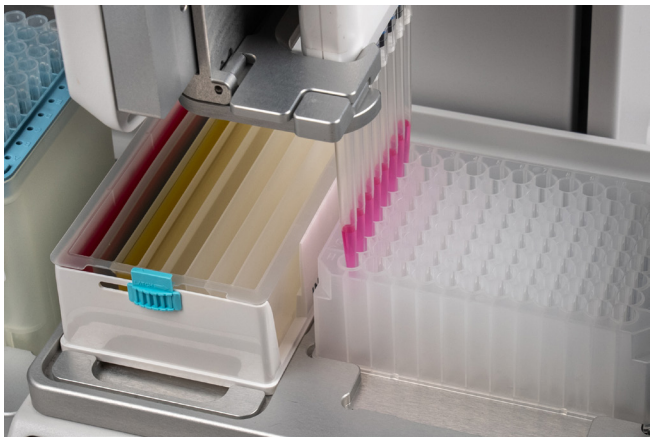
Select and run the VIALAB program 'ZymoPURE_plasmid_isolation_below_3ml' for culture inputs under 3 ml or 'ZymoPURE_plasmid_isolation_above_3ml' for 3-5 ml culture inputs (max. 5 ml). The VIAFLO on the ASSIST PLUS transfers 250 µl ZymoPURE P1 (**Figure 2**, red) from column 1 of the reservoir on position A (**Figure 4a**) to the DWP on position B (**Figure 4b**). All *E. coli* pellets are mixed 50 times at speed 10 to ensure homogeneity of different input volumes, and the VIAFLO will automatically change GRIPTIPS between columns. Afterwards, the VIAFLO will transfer 250 µl ZymoPURE P2 (**Figure 2**, blue) from the second column of the 8 row reservoir (**Figure 4a**) to the DWP on position B (**Figure 4c**). The red *E. coli* suspension with ZymoPURE P1 is mixed 10 times with ZymoPURE P2 at speed 8, until the liquid turns clear purple. The lysis is stopped with the transfer of 250 µl of ZymoPURE P3 (**Figure 2**, yellow) from the third column of the reservoir on position A (**Figure 4a**) to the DWP on position B (**Figure 5a**). Every well is mixed 13 times at speed 3 until the mixture becomes yellowish to ensure proper neutralization. The lysates, including the precipitates, are transferred from the DWP on position B to the ZymoPURE filter plate on position C (**Figure 5b**) by the VIAFLO, followed by a 5 minute incubation. The pipette instructs the operator to turn on the vacuum for 5 minutes to collect the cleared lysates.

Tip:

- The mixing cycles are increased to 13 and the mixing speed is reduced to 2 at the neutralization step with the program 'ZymoPURE_plasmid_isolation_above_3ml' to help complete neutralization.

a)

b)



c)

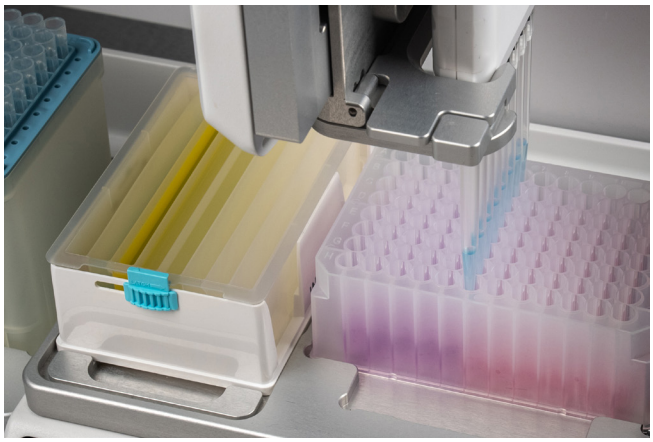


Figure 4: a) The 8 row divided reagent reservoir with base and reagents in portrait orientation on position A, and the VIAFLO transferring b) ZymoPURE P1 and c) ZymoPURE P2 to the DWP on the ASSIST PLUS.

a)

b)

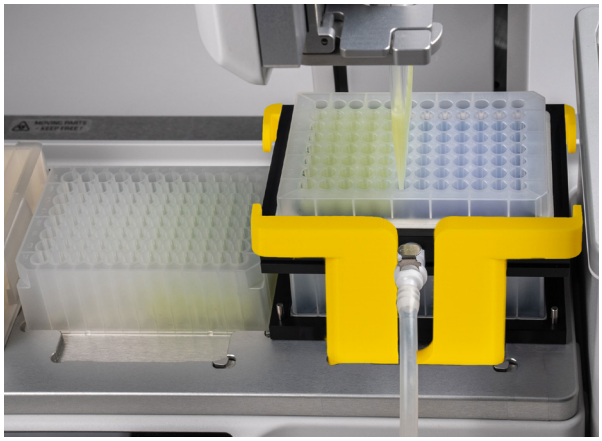


Figure 5: The VIAFLO on the ASSIST PLUS transferring a) ZymoPURE P3 to the DWP and b) the lysate to the ZymoPURE filter plate.

Step 2: **Bind plasmid DNA**

HOW TO: The operator is instructed to disassemble the EZ-Vac 96 set-up A (**Figure 3b**) and replace the empty DWP from position B with the DWP containing the cleared lysates. Discard the ZymoPURE filter plate and keep the 8 row reagent reservoir on position A (**Figure 4a**). Assemble EZ-Vac 96 set-up B by placing the manifold base, including the wash plate, on position B (**Figure 6a**), with the manifold collar and Zymo-Spin P-96 plate on top (**Figure 6b**). Connect the tubing to a collection flask.

Confirm and continue the run. The VIAFLO will transfer 220 µl of ZymoPURE binding buffer from the 4th column of the reagent reservoir on position A (**Figure 2**, light green) into every well of the DWP on position B, mixing each cleared lysate 10 times. The mixture is then transferred to the Zymo-Spin P-96 plate at position C. After incubating the lysates for 2 minutes, the pipette instructs the operator to turn on the vacuum and wait until all liquid has passed through.

Tip:

- The volume of the ZymoPURE binding buffer is reduced to 165 µl when using the VIALAB program ZymoPURE_plasmid_isolation_above_3ml to accommodate the reduced volume of cleared lysate.

a) b)

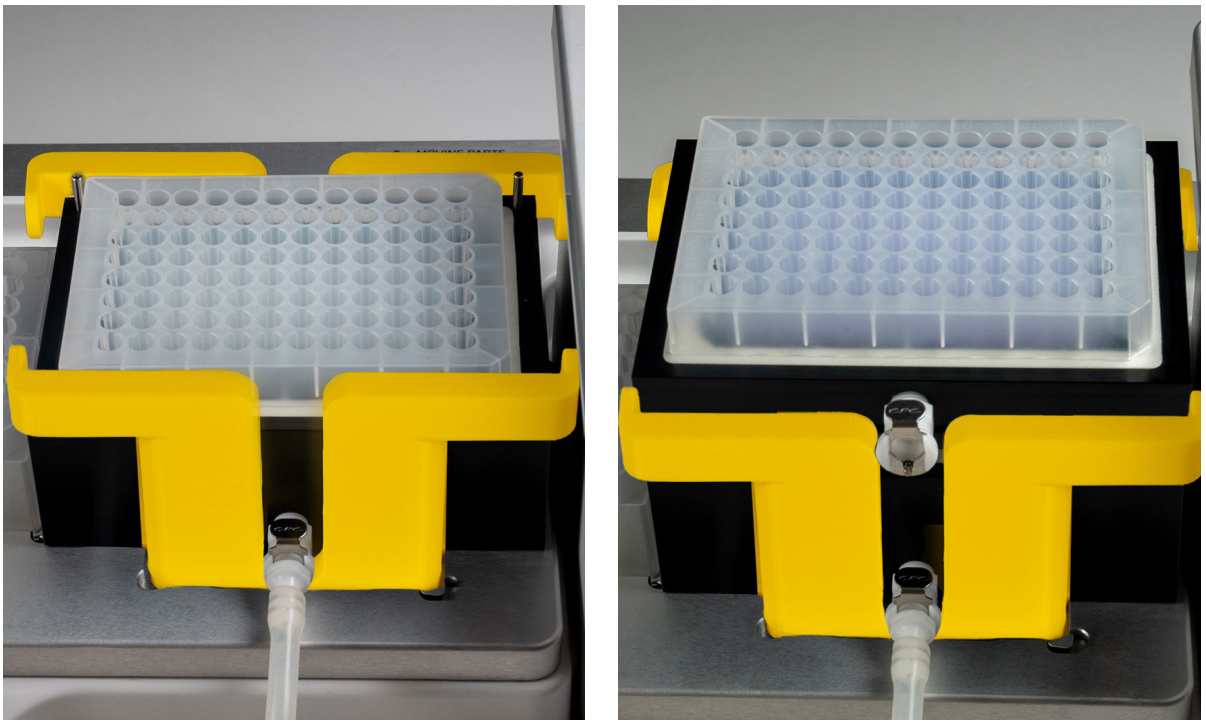


Figure 6: EZ-Vac 96 set-up B on ASSIST PLUS. a) The manifold base with the tubing connection accommodates the wash plate and is placed on deck position C. b) The manifold collar without the tubing connection is placed onto the wash plate, and accommodates the Zymo-Spin P-96 plate.

Step 3: **Wash plasmid DNA**

HOW TO: The VIAFLO instructs the operator to load another automation-friendly 8 row reagent reservoir with 27 ml of ZymoPURE wash 1 in columns 1 to 3 (**Figure 7**, lilac), 27 ml of ZymoPURE wash 2 in columns 4 to 6 (**Figure 7**, blue), 21 ml of ZymoPURE wash 2 in column 7 (**Figure 7**, blue) and 14 ml of ZymoPURE elution buffer in column 8 (**Figure 7**, green) on position A. Remove the empty DWP from position B. 800 µl of ZymoPURE wash 1 (**Figure 7**, lilac) is added to every column of the Zymo-Spin P-96 plate on position C (**Figure 7**). Once all ZymoPURE wash 1 is transferred, a prompt message instructs the operator to turn on the vacuum pump and wait until all the liquid has gone. After turning off the vacuum pump, the VIAFLO will follow the same procedure for the second wash step using 800 µl of ZymoPURE wash 2 from columns 4 to 6 of the reagent reservoir on position A (**Figure 7**, blue). Again, the operator is instructed to turn on the vacuum until all the liquid has gone, then the VIAFLO will continue with another wash step by transferring 200 µl of ZymoPURE wash 2 from column 7 of the reagent reservoir (**Figure 7**, blue) to every well on position C. The pipette instructs the operator to apply the maximum vacuum force (>600 mm Hg) for 10 minutes to dry the membrane. Disassemble the EZ-Vac 96 set-up B and blot the nozzles of the Zymo-Spin P-96 plate with clean absorbent paper to remove any residual buffer droplets.

Tip:

- A tip touch at the target plate guarantees complete droplet removal when working with ethanol-containing buffers.

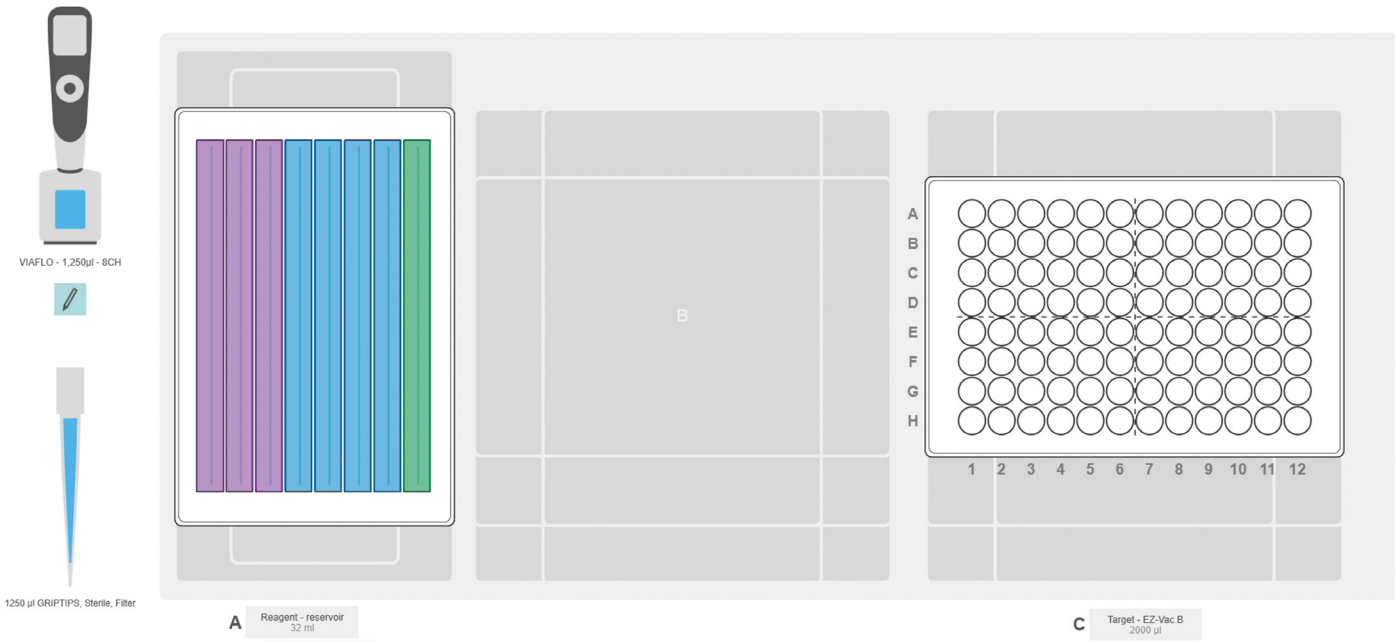


Figure 7: Deck set-up with the EZ-Vac 96 set-up B. **Position A**: Reagents – 8 row polypropylene reservoir with base (Lilac – ZymoPURE wash 1; Blue – ZymoPURE wash 2; Green – ZymoPURE elution buffer). **Position B**: Empty. **Position C**: Target – EZ-Vac 96 set-up B.

Step 4: **Elute plasmids DNA from Zymo-Spin P-96 plate**

HOW TO: Assemble EZ-Vac 96 set-up C by placing the manifold bed with a fresh DWP on position C (**Figure 3a**). Put the manifold collar with the Zymo-Spin P-96 plate on top of the DWP (**Figure 3b**), connect the tubing to the vacuum pump.

After confirmation, the VIAFLO transfers 125 µl of ZymoPURE elution buffer from the last row of the reagent reservoir on position A (**Figure 7**, green) to every column of the Zymo-Spin P-96 plate. After 2 minutes, the pipette instructs the operator to apply maximum vacuum for 30 seconds, and informs when the run is finished. Disassemble the EZ-Vac 96 set-up C and discard the Zymo-Spin P-96 plate. Seal and store the DWP containing the isolated plasmid DNA as indicated in the kit protocol.

Experiment

We compared the automated miniprep protocol for 96 replicates of 1 ml *E. coli* JM109 (pGL3) culture described above to a similar manual approach. *E. coli* JM109 (pGL3) was cultured overnight in Luria-Bertani (LB) media containing 100 µg/ml ampicillin (as indicated in the kit protocol) on a shaker at 37 °C and 250 RPM. 1 ml of culture was transferred to the provided DWP and centrifuged as indicated in the kit protocol. Additionally, 5 ml culture input was tested by pelleting with multiple centrifugation steps, using a reduced volume of the ZymoPURE binding buffer during the automated miniprep protocol.

Results

The yields and purities of eluted plasmid DNA (n=96) were measured using a Nanodrop Spectrophotometer. Total plasmid DNA yield and purified plasmid DNA were consistently high for 1 ml and 5 ml cultures, and sufficient to carry out sequencing and mammalian cell transfection. The average total plasmid DNA yield from 5 ml was about 5 times greater than the 1 ml cultures (15.3 µg from 1 ml and 69.7 µg from 5 ml) (**Figure 9**). High absorbance ratios (pure DNA has a 260/280 absorbance ratio above 1.8 and a 260/230 absorbance ratio above 2.0) also showed that the automated plasmid purification protocol was also shown to be very effective at removing proteins and unwanted chemicals from the recovered plasmid DNA. 12 samples – 1 randomly selected from each plate – were run through gel electrophoresis for 120 minutes at 120 volts on a 0.8 % TE agarose gel, which showed the consistent purification of pGL3 control plasmid (**Figure 10**). The majority of recovered plasmid was supercoiled, which is preferred for efficient transfection.

Long read nanopore sequencing validated the compatibility of plasmid DNA for whole-plasmid sequencing. Nanopore sequencing confirmed that the recovered plasmid had the correct sequence, was free from genomic DNA, and exhibited purity suitable for sensitive downstream applications (**Figure 8**).

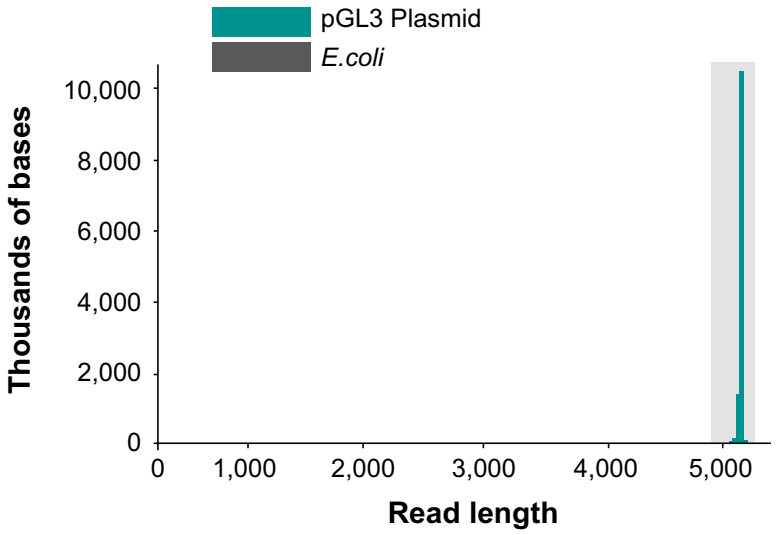


Figure 8: Read length distribution of plasmid DNA purified from 5 ml overnight *E. coli* culture using the automated ZymoPURE 96 Plasmid Miniprep protocol.

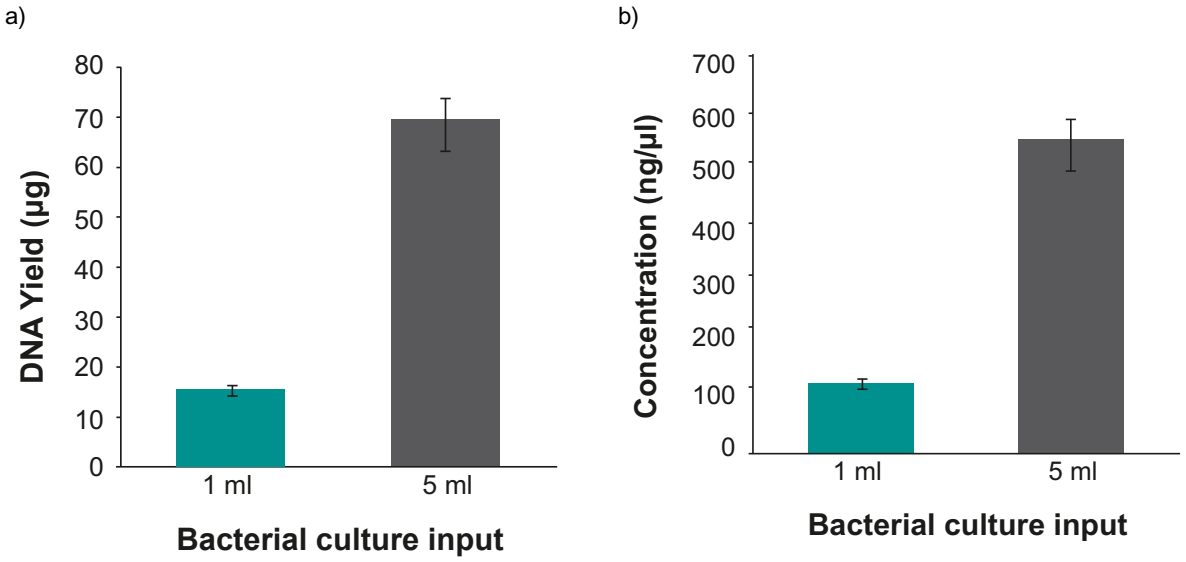


Figure 9: a) Total plasmid yield and b) concentration were consistently high for both 1 ml and 5 ml cultures.

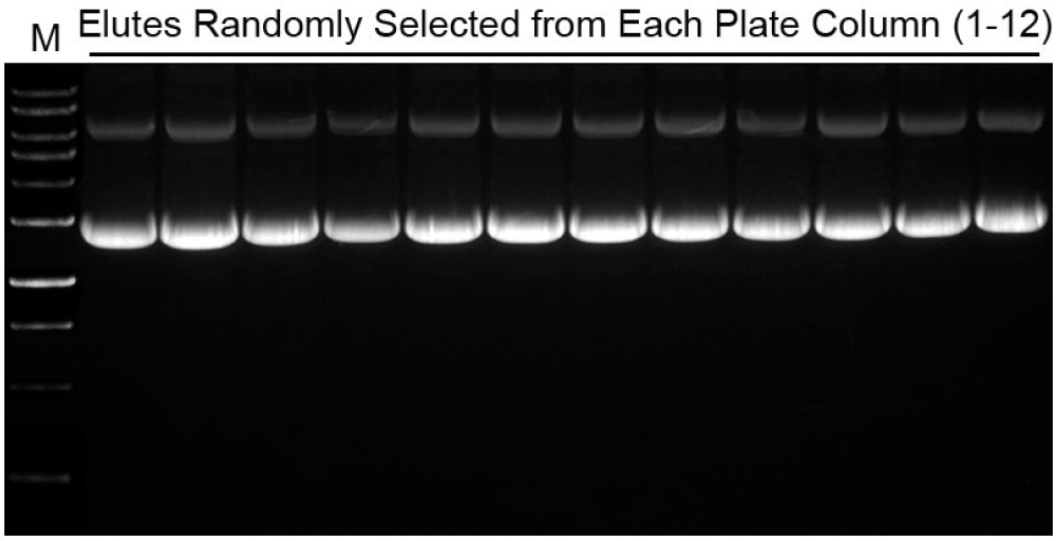


Figure 10: Agarose gel electrophoresis confirmed that the recovered plasmid was predominantly supercoiled and free of host genomic DNA and RNA.

Transfecting mammalian cells with plasmid DNA is often used in the development of modern molecular based therapies, including gene editing, recombinant protein expression, cell and gene therapy, and recombinant viral vector production. Purified pGL3 control plasmid from 5 ml of overnight culture was transfected into HEK293T cells to test the feasibility of using plasmid DNA purified with this automated method for recombinant protein expression and viral vector production. HEK293T cells were plated on a 96 well plate at 10K cells/well, and each well was transfected with 200 ng of plasmid 24 hours later using FuGENE 4K Transfection Reagent. Transfection efficiency was assessed 48 hours later by measuring luciferase expression. Average transfection efficiency was high for plasmid DNA prepared using this automated method, and consistent across the 96 well plate (**Figure 11**). Luciferase expression levels were consistent between the high throughput miniprep method and a manual, endotoxin-free, anion exchange plasmid purification method.

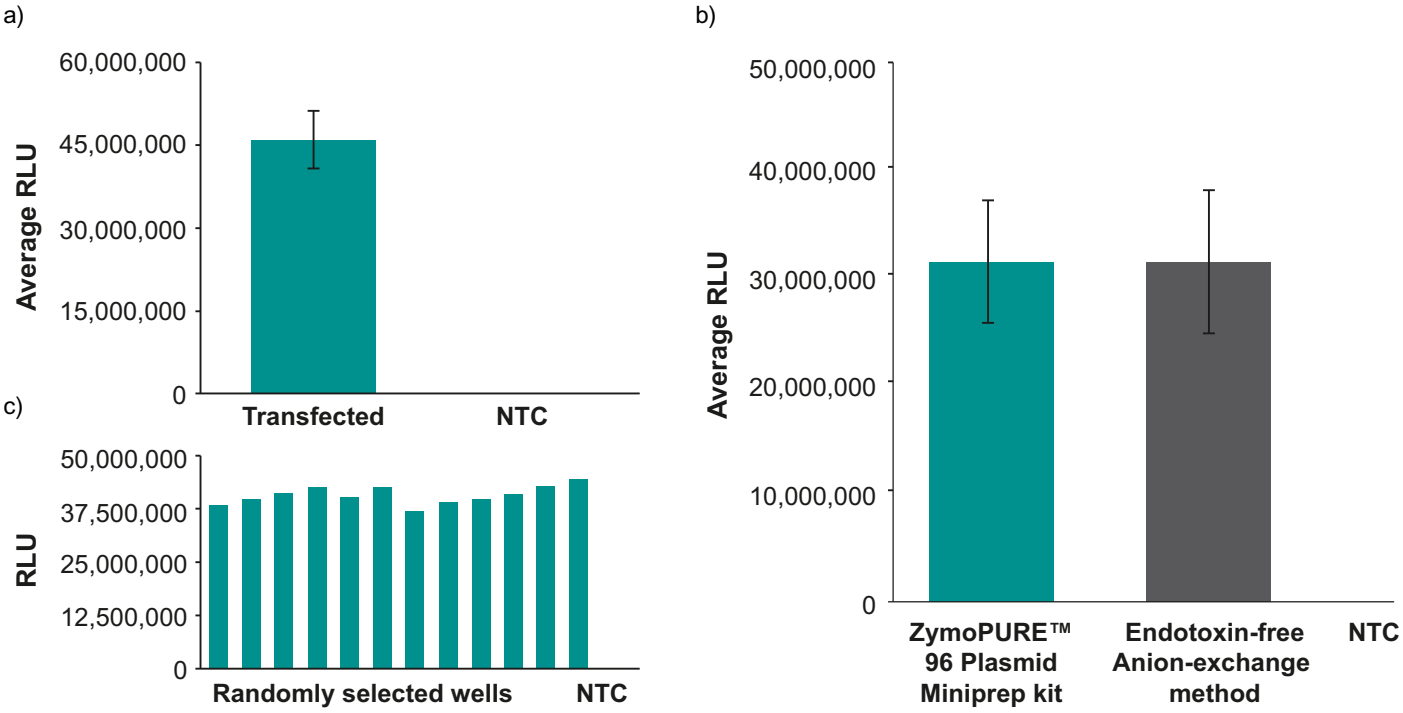


Figure 11: a) Relative light units (RLU) averaged across all transfected wells in the 96 well HEK293T culture plate and b) for randomly selected transfected wells in the 96-well HEK293T culture plate. No transfection control (NTC) wells had low RLU and plasmid transfection into HEK293T cells, resulting in consistently high luciferase expression comparable to plasmids produced with a manual, endotoxin-free, anion exchange plasmid purification method.

Remarks

- Vacuum manifold: The labware of ZymoPURE 96 Plasmid Miniprep kit can be used for any vacuum manifold using a 96 well plate format.
- VIALAB software: The VIALAB programs can be easily adapted to specific pipette, labware and protocols, for instance when partial plates are needed.
- Partial plates: Programs can be adapted at any time to a different number of samples, giving laboratories total flexibility to meet current and future demands.

Conclusion

- Ultrapure, transfection-grade plasmid DNA can be obtained using the ZymoPURE 96 Plasmid Miniprep Kit on the ASSIST PLUS pipetting robot, allowing hands-free pipetting.
- Automated plasmid purification reduces user errors and provides reliably high throughputs and yields when processing 96 samples. The VIAFLO electronic pipette on the ASSIST PLUS ensures precise and accurate liquid handling.
- The unique Zymo-Spin P-96 plate design guarantees high DNA binding capacity and rapid loading of the lysates and wash buffers.
- This protocol produces ultrapure, transfection-grade plasmid DNA suitable for downstream applications such as DNA sequencing.

Application Note

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Materials

Manufacturer	Part Number	Description	Link
INTEGRA Biosciences	4505	ASSIST PLUS pipetting robot	https://www.integra-biosciences.com/en/pipetting-robots/assist-plus
INTEGRA Biosciences	4624	50 - 1250 µl 8 channel VIAFLO electronic pipette	https://www.integra-biosciences.com/en/electronic-pipettes/viaflo
INTEGRA Biosciences	4624	1250 µl STANDARD Sterile, Filter GRIPTIPS	https://www.integra-biosciences.com/en/griptips/griptips-selector-guide
INTEGRA Biosciences	6305	300 ml Reservoir Base	https://www.integra-biosciences.com/en/reagent-reservoirs/automation-friendly-reagent-reservoirs
INTEGRA Biosciences	6374	8 row polypropylene reservoir	https://www.integra-biosciences.com/en/reagent-reservoirs/automation-friendly-reagent-reservoirs
Zymo Research	D4214	ZymoPURE 96 Plasmid Miniprep Kit	https://zymoresearch.eu
Zymo Research	S7003	EZ-Vac 96 Vacuum Manifold	https://zymoresearch.eu
Zymo Research		ASSIST PLUS adapter	Inquire at Zymo Research (1-888-882-9682 ext 3 or tech@zymoresearch.com)

Contact us: