INTEGRA

ZYMO RESEARCH

An Automated High-Throughput Solution for Purifying Transfection-Grade Plasmid DNA

Prepare transfection-grade plasmid DNA with an automated protocol using Zymo Research's ZymoPURE™ 96 Plasmid Miniprep Kit and INTEGRA's ASSIST PLUS Pipetting Robot.

Introduction

Transfecting mammalian cells with plasmid DNA has become a critical tool for studying biological processes, developing therapeutics, gene editing, producing proteins, and generating recombinant viruses. In addition, modern DNA synthesis technologies have simplified the construction of variant gene libraries for evaluating candidates of interest with transfection, but the purification of plasmid DNA that is suitable for transfecting mammalian cells remains a major bottleneck for high-throughput screening.

Traditionally, plasmid DNA that meets the quantity, concentration, and low endotoxin requirements for mammalian cell transfection is prepared from large volumes of overnight *E. coli* culture using slow gravity flow anion-exchange columns and lengthy alcohol precipitation steps, making the process poorly suited for high-throughput and automated processing.

As a result, researchers have had to resort to first isolating plasmid DNA from a few milliliters of transformed *E. coli* with a miniprep to verify the cloned sequences in the library and then performing larger scale plasmid preps suitable for transfection with the constructs that have the correct sequence. However, this entire process typically takes at least a week before the cells can be transfected with plasmids because it requires two rounds of overnight culturing, numerous plasmid purification steps, and sequencing.

Zymo Research's **ZymoPURE™ 96 Plasmid Miniprep Kit** is a high-throughput solution that is capable of recovering enough highly-concentrated, low-endotoxin plasmid for both sequencing and transfection from a single miniprep, so transfecting mammalian cells with 96 different constructs simultaneously can be accomplished in as little as a couple of days. When used with the **eight channel VIAFLO electronic pipette** and **ASSIST PLUS** pipetting robot from INTEGRA Biosciences, the number of hands-on steps is drastically reduced, resulting in an optimized, automated, and accurate transfection-grade plasmid purification workflow that is ideal for high-throughput, transfection-based screening.

INTEGRA ASSIST PLUS with the EZ-Vac 96 Vacuum Manifold on Deck

This application note details an automated miniprep protocol for plasmid purification of up to 5 ml of overnight *E. coli* culture using the **ZymoPURE™ 96 Plasmid Miniprep Kit** with the EZ-Vac 96 Vacuum Manifold and the **eight channel VIAFLO electronic pipette** with the **ASSIST PLUS** pipetting robot.

Key Benefits

- Reliably perform 96 transfection-grade plasmid minipreps simultaneously with our automated protocol using the **ZymoPURE™ 96 Plasmid Miniprep Kit, eight channel VIAFLO electronic pipette, ASSIST PLUS** pipetting robot and **the EZ-Vac 96 Vacuum Manifold**.
- The automated high-throughput miniprep protocol using the **VIAFLO electronic pipette** and **ASSIST PLUS** pipetting robot eliminates user error and guarantees precise, accurate liquid handling.
- The **ASSIST PLUS** pipetting robot is an affordable benchtop instrument for automating liquid handling that has a small footprint and includes the **VIALAB** pipette automation software for fast and easy programming.
- Patented ZymoPURE™ plasmid purification technology has a high-binding capacity that enables the user to achieve highly concentrated, transfection-grade plasmid DNA of up to 100 µg from just several milliliters of overnight *E. Coli* culture using a simple vacuum plate protocol. The purified plasmid is suitable for transfection, transformation, CRISPR, *in vitro* transcription/translation, recombinant viral production, cloning, sequencing and other sensitive downstream applications.
- The **ZymoPURE™ 96 Plasmid Miniprep Kit** includes a novel wash plate that minimizes cross-contamination between samples, ensuring accurate screening results.

Protocol

The **ASSIST PLUS**, when used with the **eight channel 1250 µl VIAFLO electronic pipette** and **1250 µl sterile filter GRIPTIPS®**, automates all liquid handling for the **ZymoPURE™ 96 Plasmid** protocol consisting of the following steps:

I. Lyse Culture Pellets & Filter Neutralized Lysates
II. Bind Plasmid DNA
III. Wash Plasmid DNA
IV. Elute Plasmid DNA

Before Starting

Add 88 ml of 95% ethanol to 23 ml ZymoPURE™ Wash 2 before use. Cultivate cell cultures and centrifuge to pellet in the provided 96-Well 2.0 mL Deep Well Plate (DWP) according to the ZymoPURE™ 96 Plasmid Miniprep Kit Protocol.

Manifold Set-Up

The **EZ-Vac 96 Vacuum Manifold** can be assembled with the **ZymoPURE™ 96 Plasmid Miniprep Kit** components in three different ways throughout the procedure (**Figure 1**). Please use the diagram as a reference for the correct manifold set-up during the protocol. Utilize the EZ-Vac 96 Vacuum Manifold ASSIST PLUS Adapter to securely align plates on **ASSIST PLUS** (**Appendix Figure S1**).

Required Materials*

- ZymoPURE™ 96 Plasmid Miniprep Kit
- EZ-Vac 96 Vacuum Manifold
- ASSIST PLUS Pipetting Robot
- 8 Channel VIAFLO Electronic Pipette
- 1250 µl GRIPTIPS® Sterile, Filter

*Please see Table S1 on page 13 in the Appendix for ordering information.

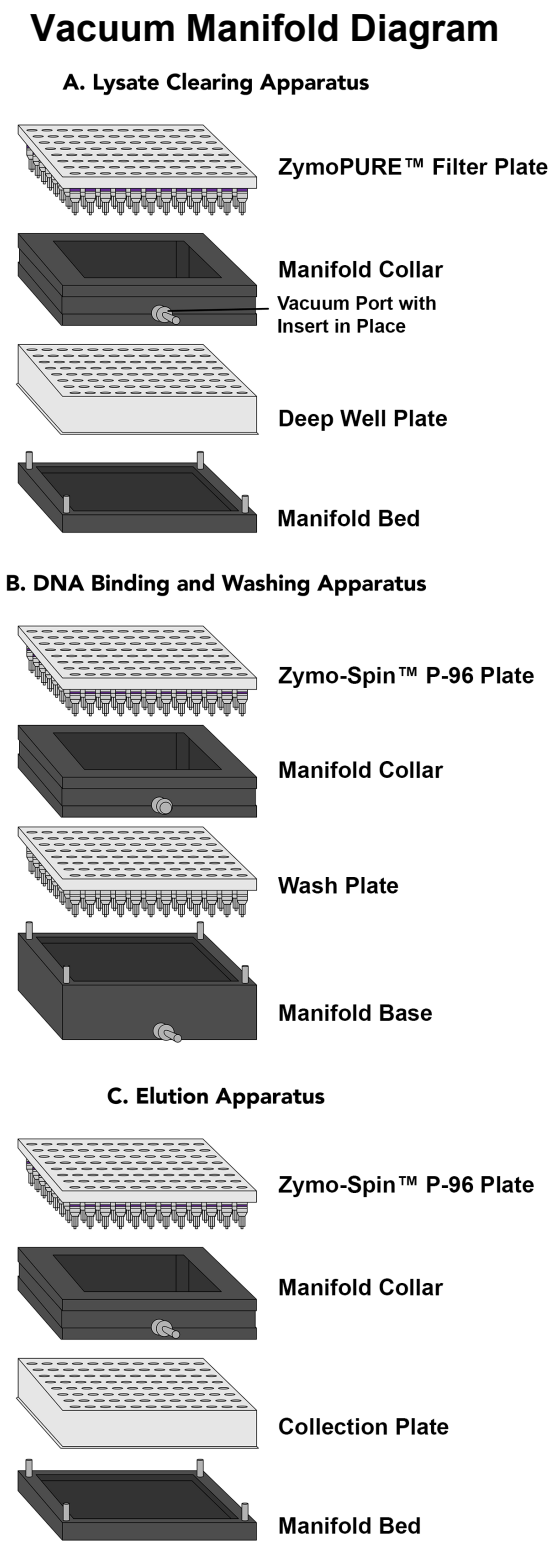

Figure 1. Diagram of the EZ-Vac 96 Vacuum Manifold setup with the ZymoPURE™ 96 Plasmid Miniprep Kit components. Refer to sections **A, B,** and **C** for the vacuum manifold and plate assembly necessary for lysate clearing, DNA binding and washing, and elution, respectively. See Appendix **Figure S1** for assembly with the ASSIST PLUS Adapter.

Step. I Lyse Culture Pellets & Filter Neutralized Lysates

1. Place an automation-friendly eight-row reservoir and base in portrait orientation on *Deck Position A* (**Figure 2**). Place the DWP that has the *E. coli* pellets in landscape orientation on *Deck Position B* (**Figure 2,** Orange).
2. **Assemble the EZ-Vac 96 Vacuum Manifold Set-up A as shown in Figure 1**

   To securely align plates on **ASSIST PLUS,** place the EZ-Vac 96 Vacuum Manifold ASSIST PLUS Adapter (Appendix, **Figure S1**) on deck before assembling manifold and plates. Place a new 96-Well 2.0 mL Deep Well Plate on top of the Manifold Bed on *Deck Position C* (**Figure 1A**). Add the Manifold Collar on top of the DWP and place a ZymoPURE™ Filter Plate on top of the Manifold Collar.
3. Load the eight-row polypropylene reservoir on Deck Position A. Fill the entire bottle volume (at least 28 ml) of each solution in the following columns:
   - ✓ ZymoPURE™ P1 in Column 1 (**Figure 2**, Red)
   - ✓ ZymoPURE™ P2 in Column 2 (**Figure 2**, Blue)
   - ✓ ZymoPURE™ P3 in Column 3 (**Figure 2**, Yellow)
   - ✓ ZymoPURE™ Binding Buffer in Column 4 (**Figure 2,** Green)
4. Select & Run appropriate VIALAB program for culture input.
   - Culture Inputs ≤ 3ml: 'ZymoPURE_plasmid_isolation_below_3 ml'
   - Culture Inputs > 3ml (max 5 ml): 'ZymoPURE_plasmid_isolation_above_ 3ml'
5. VIAFLO transfers 250 µl ZymoPURE™ P1 (**Figure 2**, Red) to the DWP on *Deck Position B*. All *E. coli* pellets are mixed ● 50 times or ■ 90 times at Speed 10 to ensure homogeneity. VIAFLO automatically changes GRIPTIPS between different columns. See **Table S2.1** for additional information on automation parameters.
6. VIAFLO transfers 250 µl ZymoPURE™ P2 (**Figure 2**, Blue) to the DWP on *Deck Position B*. The red ZymoPURE™ P1 and *E. coli* suspension is mixed 10 times at Speed 8 with the blue ZymoPURE™ P2 until the liquid turns clear purple. See **Table S2.2** for additional information on automation parameters.
7. VIAFLO transfers 250 µl ZymoPURE™ P3 (**Figure 2**, Yellow) to the DWP on *Deck Position B*. Each well is mixed ● 10 times at Speed 3 or ■ 13 times at Speed 2 until the mixture becomes yellowish, signifying complete neutralization. See **Table S2.3** for additional information on automation parameters.
8. The entire neutralized lysate, including the precipitates, is transferred by VIAFLO from the DWP on *Deck Position B* to the ZymoPURE™ filter plate on *Deck Position C*. The system will pause for 5 minutes to incubate the neutralized lysate. After five minutes, apply vacuum pressure at 300 mm Hg for five minutes to collect the cleared lysates. See **Table S2.4** for additional information on automation parameters.

The ● symbol represents the protocol to follow for bacterial culture inputs ≤ 3 ml.

The ■ symbol represents the protocol to follow for bacterial culture inputs > 3 ml.

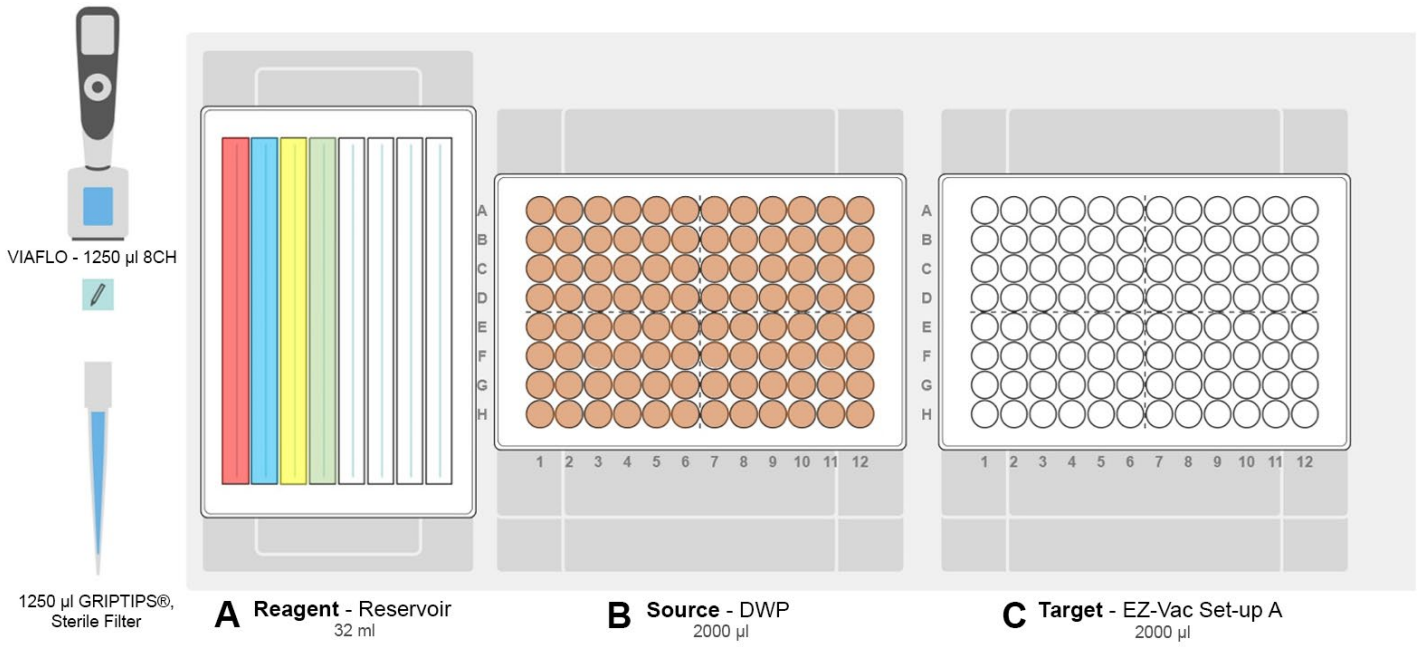

Figure 2: Deck map with EZ-Vac 96 Manifold Set-up A. **Position A:** Reagents – eight-row polypropylene reservoir with base (Red - ZymoPURE™ P1, Blue - ZymoPURE™ P2, Yellow - ZymoPURE™ P3, Green - ZymoPURE™ Binding Buffer). **Position B**: Source - Deep Well Plate (DWP) with *E. coli* pellets (Orange). **Position C**: Target EZ-Vac 96 Set-up A.

Step II. Bind Plasmid DNA

1. **Assemble the EZ-Vac 96 Vacuum Manifold Set-up B as shown in Figure 1**
   Disassemble the EZ-Vac 96 Set-up A and discard the ZymoPURE™ Filter Plate. Discard the empty DWP on *Deck Position B*. Place the DWP that contains the cleared lysates on *Deck Position B*.

   Utilize the EZ-Vac 96 Vacuum Manifold ASSIST PLUS Adapter to securely align plates on ASSIST PLUS (**Figure S1**). Place a Wash Plate on top of the Manifold Base on *Deck Position C*. Add the Manifold Collar on top of the Wash Plate and place a Zymo-Spin P-96 Plate on top of the Manifold Collar (**Figure 1B**).

2. **Confirm Correct Manifold Set-Up & Continue the Run**
   VIAFLO transfers ● 220 µl or ■ 165 µl ZymoPURE™ Binding Buffer from the fourth column of the reagent reservoir (**Figure 2**, Green) into every well of the DWP on *Deck Position B*; each cleared lysate is mixed 10 times. See **Table S2.5** for additional information on automation parameters.

   The mixture is then transferred to the Zymo-Spin P-96 Plate on *Deck Position C*. After incubating the lysates for at least 2 minutes, the pipette informs the user to apply vacuum pressure at 300 mm Hg until all liquid has passed through before proceeding. See **Table S2.6** for additional information on automation parameters.

Step III. Wash Plasmid DNA

1. Load another eight-row polypropylene reservoir in *Deck Position A*. Fill the following volumes of each solution into the specified columns:
   - 27 ml ZymoPURE™ Wash 1 in Columns 1-3 (**Figure 3**, Lilac)
   - 27 ml ZymoPURE™ Wash 2 in Columns 4-6 (**Figure 3**, Blue)
   - 21 ml ZymoPURE™ Wash 2 in Column 7 (**Figure 3**, Blue)
   - 14 ml ZymoPURE™ Elution Buffer in Column 8 (**Figure 3**, Green)
2. Discard the empty DWP on *Deck Position B*.
3. VIAFLO will add 800 µl ZymoPURE™ Wash 1 (**Figure 3**, Lilac) to every well of the Zymo-Spin P-96 Plate on *Deck Position C*. Once Wash 1 is transferred to all wells, a message will inform the user to apply vacuum pressure at 300 mm Hg until all liquid has passed through. See **Table S2.7** for additional information on automation parameters.
4. The user will be prompted by the pipette to turn the vacuum pump off. Once the vacuum is off, VIAFLO will add 800 µl ZymoPURE™ Wash 2 (**Figure 3**, Blue) to every well of the Zymo-Spin P-96 Plate on *Deck Position C*. Once Wash 2 is transferred to all wells, a message informs the user to apply vacuum pressure at 300 mm Hg until all liquid has passed through. See **Table S2.8** for additional information on automation parameters.
5. Once the user turns the vacuum pump off, VIAFLO transfers 200 µl ZymoPURE™ Wash 2 from Column 7 of the reagent reservoir (**Figure 3**, Blue) to every well of the Zymo-Spin P-96 Plate on *Deck Position C*. The pipette informs the user to apply the maximum vacuum force (>600 mm Hg) for 10 minutes to dry the membrane.
6. Disassemble 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. Discard the Wash Plate.

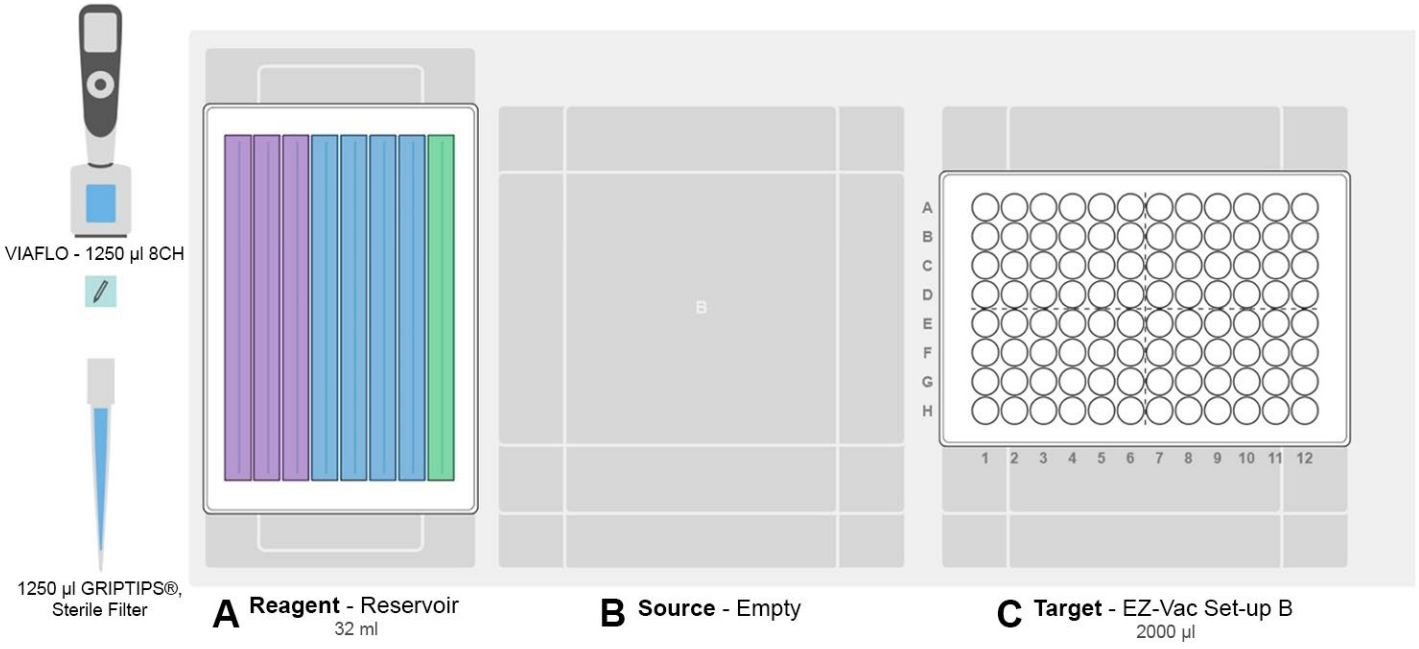

Figure 3: Deck map with EZ-Vac 96 Set-up B. **Position A:** Reagents – eight-row polypropylene reservoir with base (Lilac - ZymoPURE™ Wash 1, Blue - ZymoPURE™ Wash 2, Green - ZymoPURE™ Elution Buffer). **Position B**: Empty. **Position C**: Target - EZ-Vac 96 Set-up B.

Step IV. Elute Plasmid DNA

1. **Assemble the EZ-Vac 96 Vacuum Manifold Set-up C as shown in Figure 1**

   Utilize the EZ-Vac 96 Vacuum Manifold ASSIST PLUS Adapter to securely align plates on **ASSIST PLUS**. Place a new 96-Well 2.0 mL Deep Well Plate on top of the Manifold Bed on *Deck Position C*. Add the Manifold Collar on top of the DWP and place the Zymo-Spin P-96 Plate from the previous step on top of the Manifold Collar (**Figure 1C**).

2. **Confirm Correct Manifold Set-Up & Continue the Run**

   VIAFLO transfers 125 µl ZymoPURE™ Elution Buffer from the last row of the reagent reservoir on position A (**Figure 3**, Green) to every column of the Zymo-Spin P-96 Plate. See **Table S2.9** for additional information on automation parameters.

   After 2 minutes, the pipette requests to apply maximum vacuum force (> 600 mm Hg) for 30 seconds and informs the user when the run is complete.

   Disassemble the EZ-Vac 96 Set-up C and discard the Zymo-Spin P-96 plate. Seal and store the DWP that has the isolated plasmid DNA as indicated in the kit protocol.

Reach out to Zymo Research at (888) 882-9682 or INTEGRA Biosciences at (603) 546-0181 to receive a detailed script.

Results & Discussion

The preparation of transfection-grade plasmid DNA has historically been a significant bottleneck for high-throughput screening. The purpose of the protocol presented here is to simplify and streamline the high throughput preparation of plasmid that is suitable for transfecting mammalian cells. To assess the feasibility of using the ZymoPURE 96 Plasmid Miniprep Kit on the ASSIST PLUS to automate high-throughput plasmid purification, the protocol was carried out using a master overnight culture and the yield, purity, and transfectability of the recovered plasmid DNA was assessed across the 96 well plate. JM109 *E. coli* containing the mammalian luciferase expression plasmid pGL3®-control (Promega Corporation, Madison, WI, USA) was cultured overnight in Luria-Bertani (LB) media containing 100 µg/ml ampicillin, on a shaker at 37 °C and 250 RPM. 1 or 5 ml of overnight culture at an OD_{600} of 2.5A was pelleted in the provided deep well plate and processed using the above protocol to evaluate the procedure at the lower and upper limits of bacterial culture input.

Plasmid yield and purity were first assessed using a Nanodrop Spectrophotometer (Thermo Fisher, Waltham, MA, USA). The Nanodrop Spectrophotometer is a widely used quantification technique that measures UV absorbance to accurately quantify nucleic acids and detect the presence of protein or other contaminants in a nucleic acid sample. Total plasmid DNA yield was consistently high across the 96-well plates for both 1 ml and 5 ml culture inputs (**Figure 4A**) and more than enough plasmid DNA was recovered from a single miniprep to carry out both sequencing and mammalian cell transfection. Furthermore, the average total plasmid DNA yield from 5 ml of culture was about 5 times greater than the total plasmid DNA yield from 1 ml of culture (15.3 µg from 1 ml and 69.7 µg from 5 ml), indicating that this plasmid purification protocol is very effective at purifying plasmid DNA even at the recommended maximum culture volume input, which is important when working with lower copy number plasmids.

Similarly, the concentration of the purified plasmid DNA was consistently high for both 1- and 5-ml culture inputs across the 96-well plates (**Figure 4B**) and more than adequate for either Sanger or whole plasmid sequencing and common transfection methods for mammalian cells.

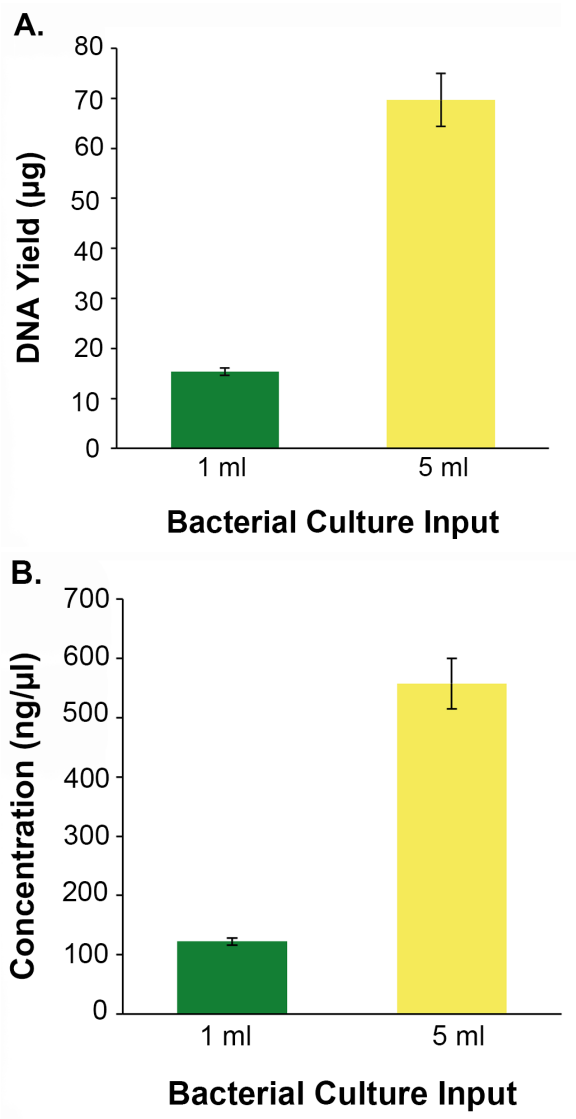

Figure 4. Total plasmid yield and concentration were consistently high for both 1- and 5-ml cultures. Average plasmid yield (A) and concentration (B) for 1- and 5-ml overnight *E. coli* culture inputs across the 96-well plates. Plasmid yield and concentration from 5 ml of culture was approximately 5 times higher than that from 1 ml of culture, demonstrating that the plasmid purification system is robust even at higher culture loads.

Host macromolecules and chemicals from the plasmid purification process can copurify with the plasmid DNA and interfere with downstream analyses and experiments, especially mammalian cell transfection. Nucleic acids absorb UV light at 260nm, whereas proteins absorb light at 280nm, and chaotropic salts, carbohydrates, phenol, and glycogen absorb light at 230nm. Therefore, the presence of these contaminants in the purified plasmid DNA can also be assessed using the Nanodrop Spectrophotometer. Generally, the ratio of absorbances at 260nm and 280nm as well as 260nm and 230nm has been used as a measure of purity in DNA. DNA that is contaminated with proteins or particular chemicals will have stronger absorbance values at 280 or 230nm, decreasing the resulting 260/280 and 260/230 ratios. Pure DNA will have a 260/280 absorbance ratio above 1.8 and a 260/230 absorbance ratio above 2.0. The plasmid purified using this automated method was found to consistently have absorbance ratios above these thresholds for both 1 ml and 5 ml culture inputs, demonstrating that this plasmid purification protocol is very effective at removing proteins and unwanted chemicals from the recovered plasmid DNA (**Figure 5A & B**).

Although the Nanodrop Spectrophotometer is regularly used to quantify nucleic acids, it cannot distinguish between RNA, host genomic DNA, and plasmid. However, the presence of RNA and host genomic DNA will make it difficult to accurately quantify plasmid DNA, which is critical to the success of downstream transfection experiments. Therefore, gel electrophoresis was used to visualize the presence of host genomic DNA and RNA in the eluted plasmid DNA, and to verify which plasmid isoforms were predominant in the eluate. The 5 ml run was selected because it is the maximum culture input the system can handle and with increased culture input there is a higher chance of copurifying host genomic DNA and RNA because of overloading the system. Twelve samples, one randomly selected from each plate column, were run for 120 minutes at 120 Volts on a 0.8% TE agarose gel. The visualized

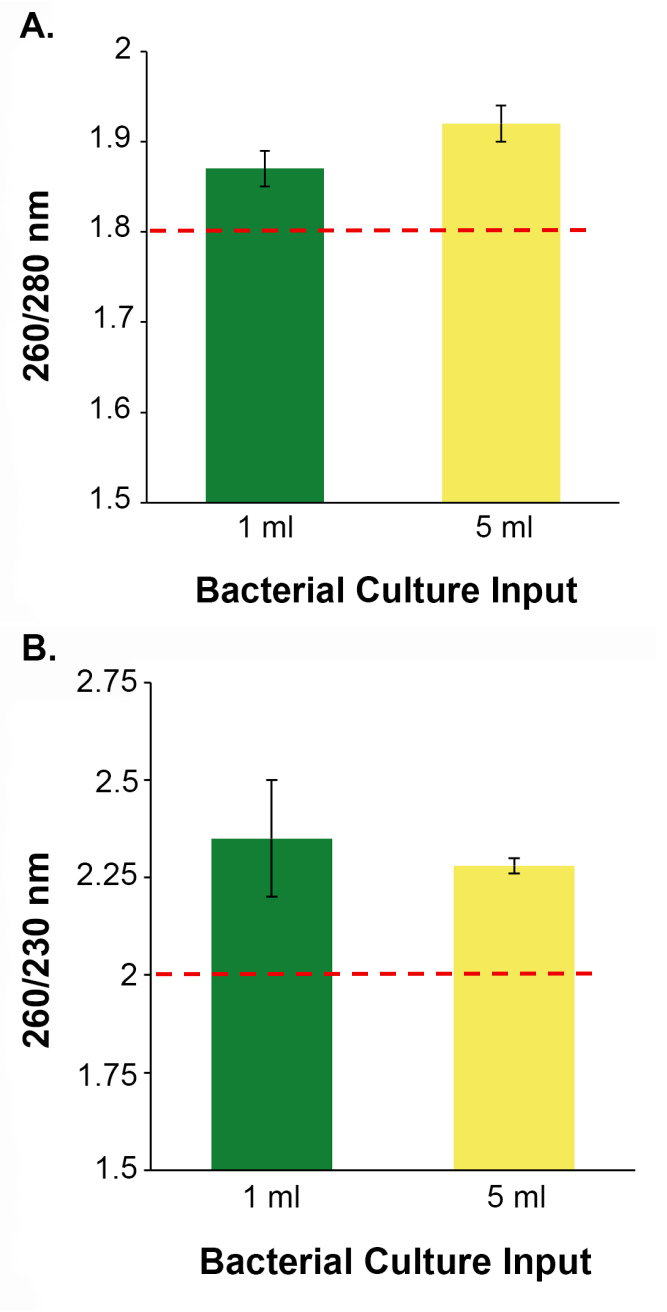

Figure 5. The isolated plasmid DNA was consistently pure for both 1- and 5-ml cultures. Average A260/A280 (A) and A260/A230 (B) ratios for 1- and 5-ml overnight *E. coli* culture inputs across the 96-well plates. A A260/A280 ratio above 1.8 indicates no protein contamination and an A260/A230 ratio above 2.0 indicates no salt contamination in the purified plasmid. These thresholds are represented by the red dotted lines in the graphs above. The A260/A280 (**A**) and A260/A230 (**B**) ratios revealed that plasmid purified from both 1- and 5-ml culture inputs were not contaminated with proteins or other chemicals such as chaotropic salts.

gel confirms the consistent purification of pGL3®-control plasmid, with no genomic DNA or RNA bands above or below the expected band of the purified plasmid DNA. In addition, the majority of the recovered plasmid migrated further than the true size of the plasmid, indicating that the purified plasmid is predominantly supercoiled, which is preferred because it is compact and can be easily taken up by cells during transfection (**Figure 6**).

Plasmid constructs are typically sequenced following purification to verify the construct contains the correct sequence of interest before performing any downstream experiments. Historically, this has typically been carried out using Sanger sequencing. However, because of Sanger sequencing's limitation in reading long DNA, multiple rounds of sequencing using different primers are necessary to sequence the entire plasmid insert. Therefore, newer long-read sequencing technologies have rapidly become a more attractive alternative to Sanger sequencing because the whole plasmid can be sequenced in one run without the need for primers and both *E. coli* genomic DNA and plasmid DNA multimers can be detected. Plasmid DNA purified using the automated method presented here was sent out for long read nanopore sequencing to validate the compatibility of recovered plasmid DNA with whole plasmid DNA sequencing. Sequencing data confirmed that the recovered plasmid had the correct sequence, was free from *E. coli* genomic DNA (**Figure 7A**), and pure enough for whole plasmid sequencing using Oxford Nanopore Technology (**Figure 7B**).

Transfecting mammalian cells with plasmid DNA has become essential for not only understanding mammalian systems, but also the development of modern molecular based therapies. Everything from gene editing, recombinant protein expression, cell and gene therapy to recombinant viral vector production uses plasmid DNA transfection. To test the feasibility of using plasmid DNA purified with this automated method for these common downstream applications, purified pGL3®-control plasmid from 5 ml of overnight culture was transfected into

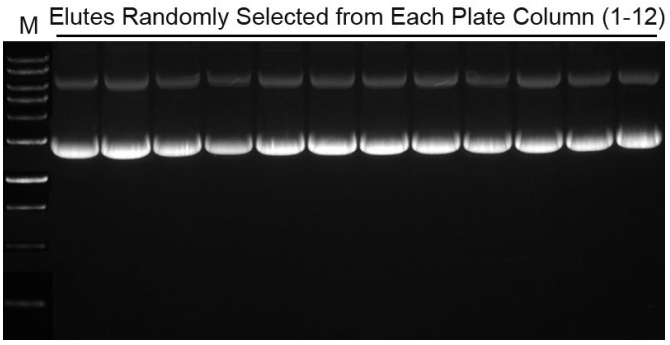

Figure 6. High quality plasmid DNA is consistently purified across the 96-well plate using the ZymoPURE™ 96 Plasmid Miniprep Kit on ASSIST PLUS. Visualization of the purified plasmid post agarose gel electrophoresis confirmed that the recovered plasmid was predominantly supercoiled and free of host genomic DNA and RNA. M: 1 kb DNA Ladder (Zymo Research).

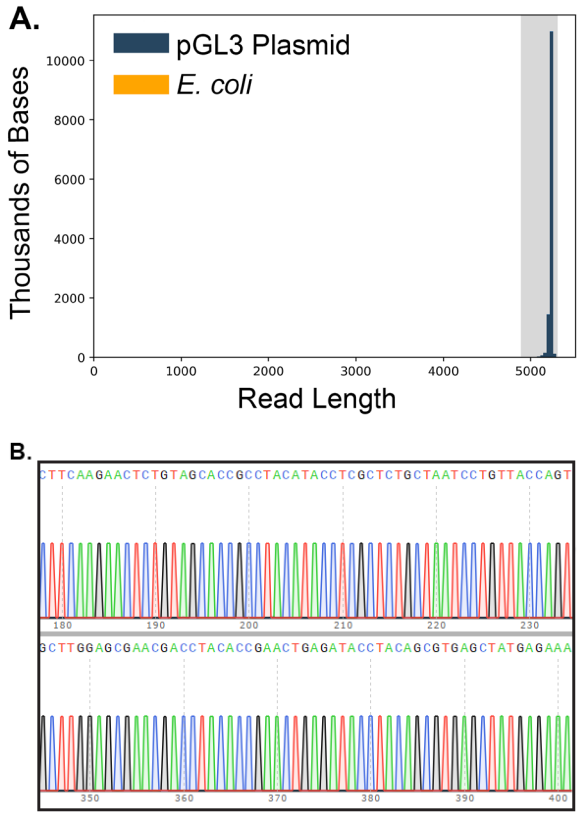

Figure 7. Plasmid DNA recovered using the ZymoPURE™ 96 Plasmid Miniprep Kit on ASSIST PLUS is free of *E. coli* host DNA and suitable for whole plasmid sequencing using nanopore technology. An example of a read length histogram (**A**) and a sequencing chromatogram (**B**) (Nanopore Sequencing) for plasmid DNA purified from 5 ml of overnight *E. coli* culture.

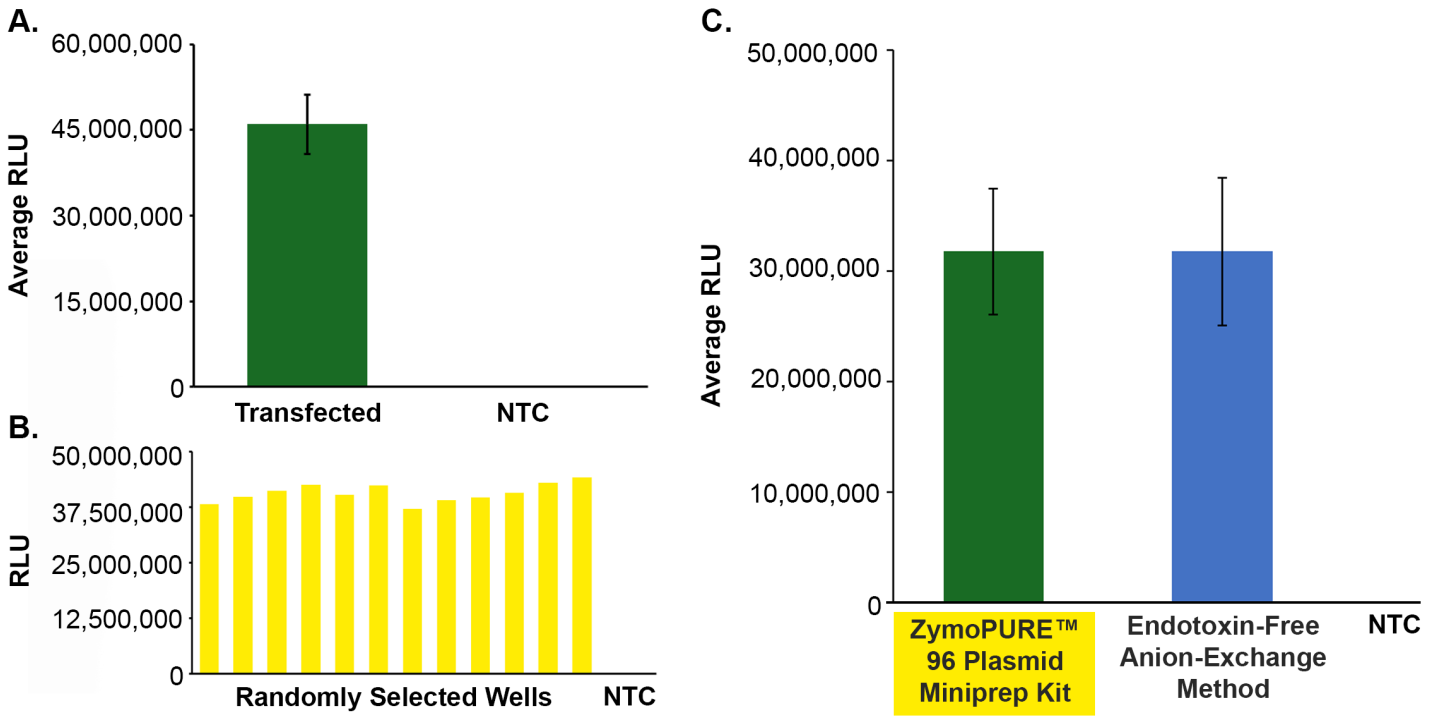

Figure 8. Plasmid DNA recovered using the ZymoPURE™ 96 Plasmid Miniprep Kit on ASSIST PLUS is suitable for mammalian cell transfection and comparable to traditional anion-exchange endotoxin-free plasmid prep methods. HEK293T cells (Passage 11) were plated on a 96-well TC plate at 10K cells/well and incubated 24 hours prior to transfection (70 % confluency). Bar graph representations of relative light units (RLU) averaged across all transfected wells in the 96-well HEK293T culture plate (**A**) and for randomly selected transfected wells in the 96-well HEK293T culture plate (**B**). *NTC (No Transfection Control) wells had low RLU and plasmid transfection into HEK293T cells resulted in consistently high luciferase expression (**A, B, & C**) that was comparable to plasmid produced with a manual anion-exchange endotoxin-free plasmid purification method (**C**).

HEK293T cells, which is a commonly used cell line for recombinant protein expression and viral vector production (**Figure 8**). HEK293T cells (Passage 11) were plated on a 96-well TC plate at 10K cells/well and each well was transfected with 200 ng of plasmid 24 hours later using FuGENE 4K Transfection Reagent (Fugent Corporation, Madison, WI, USA) following the manufacturer's suggested protocol. An additional transfection experiment was also performed with cells plated in another 96-well TC plate using plasmid prepared with this high-throughput miniprep protocol and plasmid prepared from 100 ml of overnight culture using a common manual based anion-exchange endotoxin-free plasmid maxiprep method to compare transfection efficiencies. Transfection efficiency was assessed 48 hours later by measuring luciferase expression using the One-Glo™ Luciferase Assay (Promega Corporation, Madison, WI, USA) and Veritas™ Microplate Luminometer (Promega Corporation, Madison, WI, USA). Average transfection efficiency was high for plasmid DNA prepared using this automated method and consistent for plasmid DNA purified across the 96-well plate (**Figure 8A & 8B**). Furthermore, plasmid DNA purified using this high throughput miniprep method produced luciferase expression levels that were similar to plasmid DNA purified using a manual anion-exchange endotoxin-free plasmid purification method (**Figure 8C**).

Conclusion

- **The ZymoPURE™ 96 Plasmid Miniprep Kit** used on the **ASSIST PLUS** pipetting robot enables consistent and robust recovery of highly concentrated plasmid DNA from up to 5 ml of culture input per well.
- Nanodrop absorbance ratios revealed that plasmid purified using this protocol is free of proteins and other chemicals, such as chaotropic salts.
- The recovered plasmid DNA is predominantly supercoiled and free of *E. coli* host DNA and RNA.
- The resulting plasmid DNA is pure enough for even the most sensitive downstream applications, such as whole plasmid sequencing and mammalian cell transfection.

Appendix

Table S1. Required Equipment

Zymo Research

Product	Cat. No.	Size	
ZymoPURE™ 96 Plasmid Miniprep Kit	D4214 D4215	2 x 96 4 x 96	
EZ-Vac 96 Vacuum Manifold	S7003	1 Manifold	

INTEGRA

Product	Cat. No.	Size	
ASSIST PLUS Pipetting Robot	4505	1 Unit	
8 Channel VIAFLO Electronic Pipette	4624	1 Unit	
1250 µl GRIPTIPS® Sterile, Filter	6445	5 x 96 Tips	

Figure S1. – Image of EZ-Vac 96 Manifold Set-Up with the yellow ASSIST PLUS Adapter designed to securely align plates on ASSIST PLUS. Inquire with Zymo Research at **1-888-882-9682 ext. 3** or tech@zymoresearch.com to receive an ASSIST PLUS adapter.

Table S2. – Program parameters at different steps of ZymoPURE 96 Miniprep automation protocol. *Reach out to Zymo Research at (888) 882-9682 or INTEGRA Biosciences (603) 546-0181 to receive detailed script information.*

*Add a tip touch at the target plate to guarantee complete droplet removal when working with ethanol buffers.

S2.1 - Pellet Resuspension in ZymoPURE P1 Buffer*

Transfer Speeds		Transfer Target Details		Target Mixing	
Aspiration Speeds	6/10	Target Pipetting Height	6.0 mm	Mix Volume	200 µl
Dispense Speeds	6/10	Target Safety Bottom Offset	1.5 mm	Mix Number	● 50 cycles or ■ 90 cycles
Pre-Wetting	No	Tip Travel	No	Mix Speed	10/10
Movement Speeds (X,Y,Z)	10,10,10			Mix Pipetting Height	6.0 mm
Delay (Aspirate, Dispense)	0,0			Mix Safety Bottom Offset	1.5 mm
Exit Liquid Slowly (Aspirate, Dispense)	No, No			Tip Travel	No

S2.2 - Cell Lysis in ZymoPURE P2 Buffer*

Transfer Speeds		Transfer Target Details		Target Mixing	
Aspiration Speeds	6/10	Target Pipetting Height	6.3 mm	Mix Volume	400 µl
Dispense Speeds	6/10	Target Safety Bottom Offset	2.0 mm	Mix Number	10 Cycles
Pre-Wetting	No	Tip Travel	Yes	Mix Speed	8/10
Movement Speeds (X,Y,Z)	10,10,10			Mix Pipetting Height	11.2 mm
Delay (Aspirate, Dispense)	0,0			Mix Safety Bottom Offset	2.0 mm
Exit Liquid Slowly (Aspirate, Dispense)	No, No			Tip Travel	Yes

S2.3 - Neutralization in ZymoPURE P3 Buffer*

Transfer Speeds		Transfer Target Details		Target Mixing	
Aspiration Speeds	6/10	Target Pipetting Height	10.0 mm	Mix Volume	500 µl
Dispense Speeds	6/10	Target Safety Bottom Offset	2.0 mm	Mix Number	● 10 cycles or ■ 13 cycles
Pre-Wetting	No	Tip Travel	Yes	Mix Speed	● 3/10 or ■ 2/10
Movement Speeds (X,Y,Z)	10,10,10			Mix Pipetting Height	15.0 mm
Delay (Aspirate, Dispense)	0,0			Mix Safety Bottom Offset	2.0 mm
Exit Liquid Slowly (Aspirate, Dispense)	No, No			Tip Travel	Yes

S2.4 - Transferring Lysates to ZymoPURE Filter Plate

Transfer Speeds		Transfer Source Details		Transfer Target Details	
Transfer Volume	850 µl	Source Pipetting Height	15.3 mm	Target Pipetting Height	82.1 mm
Aspiration Speeds	2/10	Source Safety Bottom Offset	0.5 mm	Target Safety Bottom Offset	2.0 mm
Dispense Speeds	5/10	Tip Travel	Yes	Tip Travel	No
Pre-Wetting	No				
Movement Speeds (X,Y,Z)	10,10,10				
Delay (Aspirate, Dispense)	0,0				
Exit Liquid Slowly (Aspirate, Dispense)	Yes, No				

S2.5 - Adding ZymoPURE Binding Buffer

Transfer Speeds		Transfer Target Details		Target Mixing	
Aspiration Speeds	6/10	Target Pipetting Height	14.8 mm	Mix Volume	500 µl
Dispense Speeds	6/10	Target Safety Bottom Offset	2.0 mm	Mix Number	10 Cycles
Pre-Wetting	No	Tip Travel	No	Mix Speed	8/10
Movement Speeds (X,Y,Z)	10,10,10			Mix Pipetting Height	● 19.0 mm or ■ 15.0 mm
Delay (Aspirate, Dispense)	0,0			Mix Safety Bottom Offset	2.0 mm
Exit Liquid Slowly (Aspirate, Dispense)	No, No			Tip Travel	Yes

S2.6 - Transferring Binding Mixture to Zymo-Spin P-96 Plate

Transfer Speeds		Transfer Source Details		Transfer Target Details	
Transfer Volume	● 920 µl or ■ 890 µl	Source Pipetting Height	18.0 mm	Target Pipetting Height	86.0 mm
Aspiration Speeds	6/10	Source Safety Bottom Offset	0.5 mm	Target Safety Bottom Offset	2.0 mm
Dispense Speeds	6/10	Tip Travel	Yes	Tip Travel	Yes
Pre-Wetting	No				
Movement Speeds (X,Y,Z)	10,10,10				
Delay (Aspirate, Dispense)	0,0				
Exit Liquid Slowly (Aspirate, Dispense)	No, No				

S2.7 - Washing with ZymoPURE Wash 1

Transfer Speeds		Transfer Target Details	
Aspiration Speed	6/10	Target Pipetting Height	96.0 mm
Dispense Speed	6/10	Target Safety Bottom Offset	2.0 mm
Pre-Wetting	No	Tip Travel	No
Movement Speeds (X,Y,Z)	10,10,10		
Delay (Aspirate, Dispense)	0,0		
Exit Liquid Slowly (Aspirate, Dispense)	No, No		

S2.8 - Washing with ZymoPURE Wash 2

Transfer Speeds		Transfer Target Details	
Aspiration Speed	6/10	Target Pipetting Height	96.0 mm
Dispense Speed	6/10	Target Safety Bottom Offset	2.0 mm
Pre-Wetting	No	Tip Travel	No
Movement Speeds (X,Y,Z)	10,10,10		
Delay (Aspirate, Dispense)	0,0		
Exit Liquid Slowly (Aspirate, Dispense)	No, No		

S2.9 Elution with ZymoPURE Elution Buffer

Transfer Speeds		Transfer Target Details	
Aspiration Speed	8/10	Target Pipetting Height	85.9 mm
Dispense Speed	8/10	Target Safety Bottom Offset	2.0 mm
Pre-Wetting	No	Tip Travel	Yes
Movement Speeds (X,Y,Z)	10,10,10		
Delay (Aspirate, Dispense)	0,0		
Exit Liquid Slowly (Aspirate, Dispense)	No, No		

Notes