Application Note

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INTEGRA

Plasmid DNA purification of MACHEREY-NAGEL's NucleoSpin® 96 Plasmid kit and NucleoVac 96 Vacuum Manifold

Introduction

The use of silica membrane-based DNA purification kits is a convenient way to prepare plasmid DNA samples for cloning or subsequent analysis, e.g. sequencing or restriction analysis. MACHEREY-NAGEL's NucleoSpin 96 Plasmid kit is designed for high throughput purification of high-copy plasmid DNA from *E. coli* in a 96 well plate format. The alkaline lysis-based miniprep protocol typically yields 5-15 µg of plasmid DNA from 1.5 ml overnight cultures. However, it is a time-consuming step in genetic analysis. Automation of the protocol on an ASSIST PLUS pipetting robot with a VIAFLO 12 channel 1250 µl electronic pipette offers an easy and efficient way to extract and purify plasmid DNA with minimal hands-on time, guaranteeing perfect and reproducible liquid handling while protecting the user from repetitive strain injuries.

Key benefits:

- MACHEREY-NAGEL's NucleoSpin 96 Plasmid kit and NucleoVac 96 Vacuum Manifold are a proven method for high throughput plasmid DNA purification.
- Automation of the pipetting steps of the miniprep workflow with the ASSIST PLUS pipetting robot allows for more hands-free time for the user and increased reproducibility.
- 96 samples can be purified in less than 1 hour by processing 12 samples in parallel. In combination with the ASSIST PLUS, the VIAFLO electronic pipettes provide unmatched pipetting ergonomics, eliminating the need to hold the pipette during the pipetting steps.
- The ASSIST PLUS performs all the pipetting steps of the protocol and guides the user through each manual intervention.

Step-by-step procedure:

Experimental set-up

The ASSIST PLUS pipetting robot is used to automate the pipetting steps of the MACHEREY-NAGEL NucleoSpin 96 Plasmid kit protocol for plasmid DNA purification. The liquid handling platform guides the user whenever manual interventions are required during the process. The following procedure is an example based on the kit manufacturer's protocol for purification of 96 samples.

Bacteria are first cultivated at 37 °C following MACHEREY-NAGEL's recommendations, either in a MACHEREY-NAGEL Culture Plate (Square-well Block) or in tubes. For the current protocol, the bacterial cultures are grown in tubes and transferred into a Culture Plate. Centrifuge the bacterial cultures for 10 min at 1000 x g to pellet the bacteria. Discard the supernatant and remove the residual medium by tapping the plate upside down on a clean paper sheet or soft tissue. The ASSIST PLUS pipetting robot operates a VIAFLO 12 channel 1250 µl electronic pipette with 1250 µl Sterile, Filter GripTips.

ASSIST PLUS

Prepare the deck of the pipetting robot as follows (**Figure 1**):

Deck position A: 8 row reagent reservoir containing the different buffers for the protocol.
Deck position B: 96 well Culture Plate containing the centrifuged bacterial cells, placed on the Teleshake microplate shaker in portrait orientation.
Deck position C: NucleoVac 96 Vacuum Manifold containing and/or supporting the different 96 well plates. The vacuum manifold needs to be placed on the instrument in portrait orientation.

Important:
Align the Teleshake and vacuum manifold before each run (see appendix).

The plasmid DNA purification protocol is performed using a pipetting program generated with the VIALAB software. The ASSIST PLUS liquid handling platform guides the user through each step of the protocol.

Figure 1: General set-up for the plasmid DNA purification program. **Position A:** 8 row reagent reservoir. **Position B:** Culture Plate (96 Square-well Block) placed on the Teleshake microplate shaker. **Position C:** NucleoVac 96 Vacuum Manifold.

Overview of the extraction and purification steps:
1. Resuspension of the bacterial cells
2. Lysis of the bacterial cells
3. Neutralization
4. Lysate clearing
5. DNA binding
6. Washing and drying
7. Elution

For the initial set-up, assemble the manifold as described in **Figure 2**, with the NucleoSpin Plasmid Filter Plate (violet rings) on top of the manifold and the NucleoSpin Binding Plate (white rings) inside.

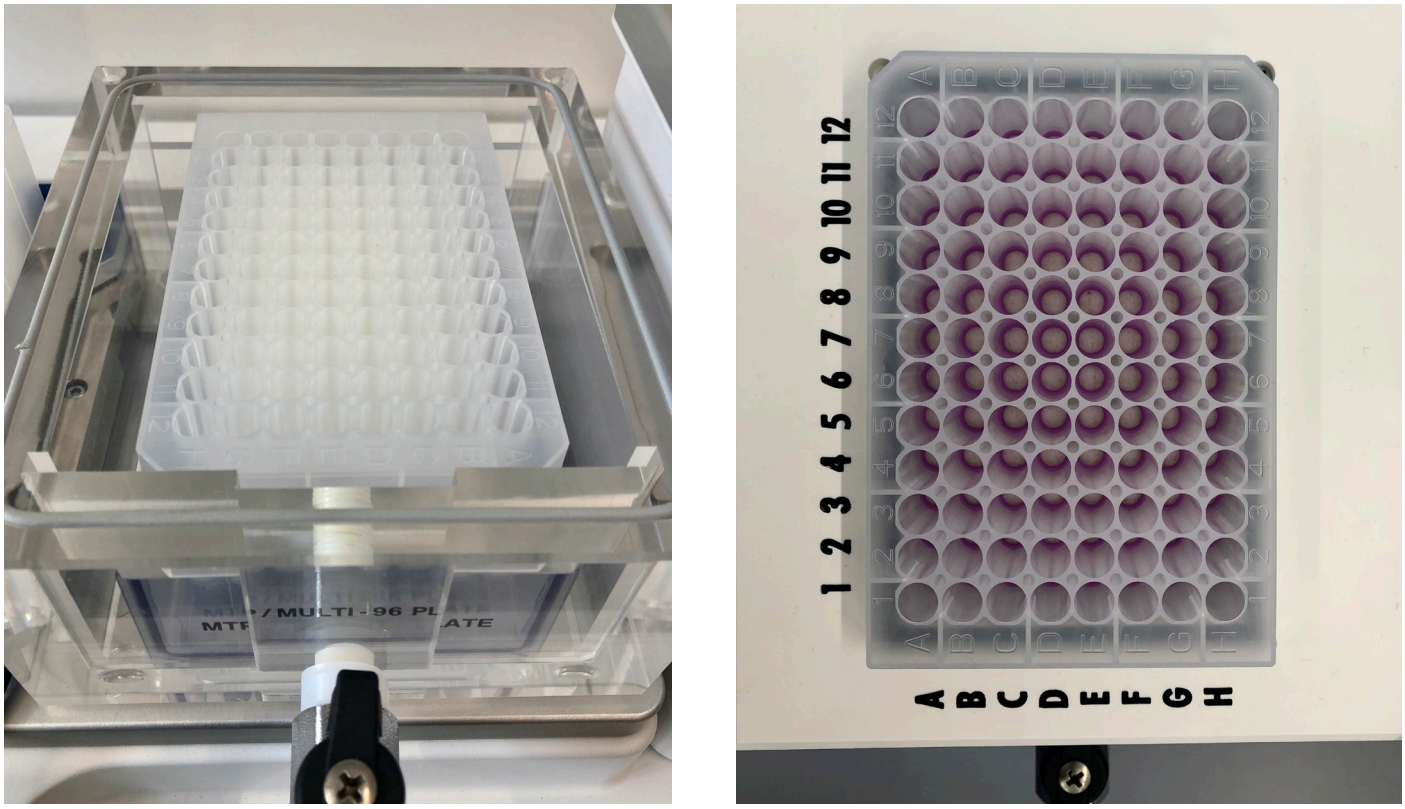

Figure 2: Vacuum manifold set-up for steps 1 to 4. Insert the MTP/Multi-96 Plate spacer, waste container and NucleoSpin Binding Plate (white rings, left) in the manifold. Place the manifold lid on top of the manifold base, and then place the NucleoSpin Plasmid Filter Plate (violet rings, right) on top of the manifold.

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1. Resuspension of the bacterial cells

STEP: Resuspend the harvested bacterial cells.

HOW TO: Fill the 8 row reagent reservoir with the different buffers as described in **Figure 3**. Select and run the VIALAB program ‘MN Plasmid’. The ASSIST PLUS transfers 250 µl of Resuspension Buffer A1 from row A of the reservoir into the Culture Plate using the Repeat Dispense mode. Turn on the shaker as indicated by the pipette and resuspend the cells by shaking at 1000 rpm. The VIALAB program includes a 4 minute delay, after which the pipette informs the user to stop shaking the plate.

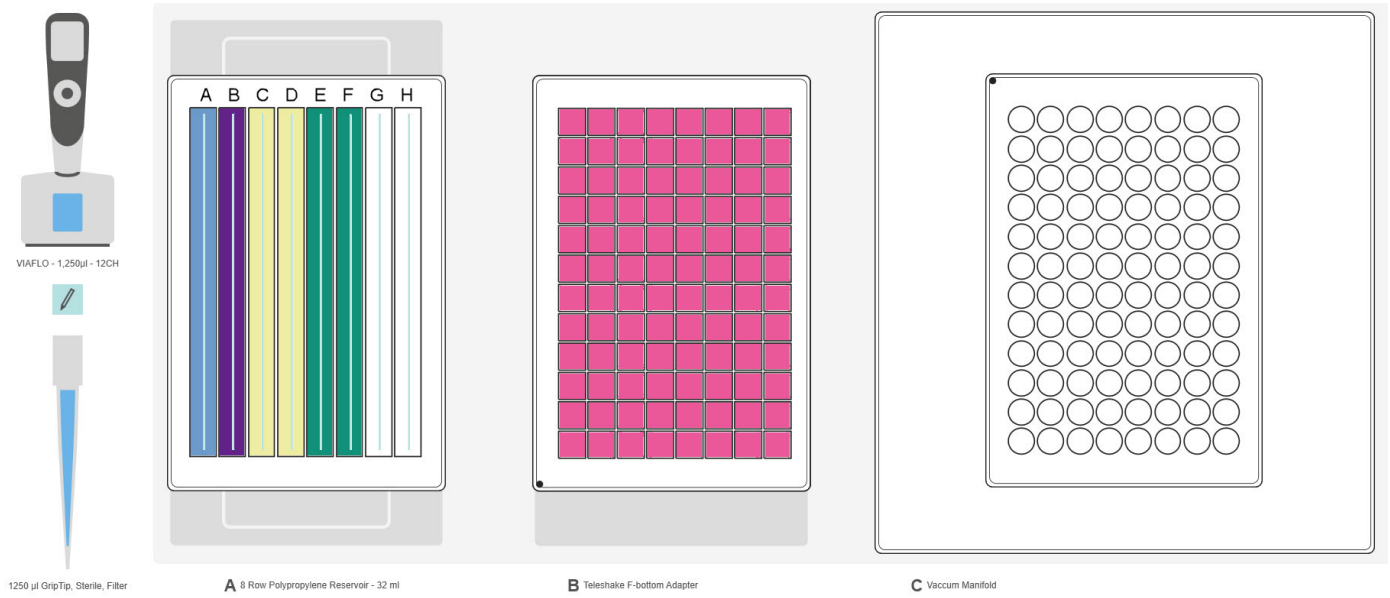


Figure 3: Set-up for the plasmid DNA purification program. **Position A**: 8 row reagent reservoir filled with Resuspension Buffer A1 in row A (blue, 25 ml), Lysis Buffer A2 in row B (purple, 26 ml), Neutralization Buffer A3 in rows C and D (yellow, 18 ml) and Wash Buffer AW in rows E and F (green, 30 ml). **Position B**: Culture Plate containing the centrifuged bacterial cells for resuspension (pink), placed on the Teleshake. **Position C**: NucleoVac 96 Vacuum Manifold set up as described in **Figure 2**.

		Tips: • Adding an air gap at the end of the aspiration step eliminates droplet build-up after the liquid dispense and avoids the use of a tip touch to prevent any risk of cross-contamination. • For the same reason, the ASSIST PLUS also dispenses the buffer from the upper part of the wells without tip travel. • Adding a post-dispense ensures the accuracy of each dispense when using the Repeat Dispense mode.
2. Lysis of the bacterial cells	**STEP:** Lyse the suspended bacterial cells.	**HOW TO:** The ASSIST PLUS transfers 250 µl of Lysis Buffer A2 from row B of the reservoir into the cell culture plate using the Repeat Dispense function. Turn on the shaker as indicated by the pipette and incubate at room temperature with moderate shaking (300 rpm). A 2 minute delay is set in the VIALAB program, after which the pipette informs the user to stop shaking the plate. The program directly continues with the next step.
3. Neutralization	**STEP:** Neutralize the lysis buffer.	**HOW TO:** The ASSIST PLUS pipetting robot adds 350 µl of Neutralization Buffer A3 to the suspension using the Repeat Dispense mode.

4. Clearing of the crude lysates	**STEP:** Clear the crude lysates by vacuum filtration using the NucleoSpin Plasmid Filter Plate.	**HOW TO:** The suspension is mixed three times by pipetting up and down, and then the entire volume is transferred to the NucleoSpin Plasmid Filter Plate. A 1 minute delay is set to allow room temperature incubation for optimal precipitation. The pipette prompts the user to turn on the vacuum pump. Apply a vacuum of -0.2 to -0.4 bar and adjust it to establish a flow rate of 1-2 drops per second (this takes approx. 4 minutes, including a delay set up in the VIALAB program). When the crude lysate has passed the NucleoSpin Plasmid Filter Plate, release the vacuum as indicated by the pipette. **Tips:** • The use of Wide Bore GripTips may prevent shearing of DNA when transferring the crude lysate to the NucleoSpin Plasmid Filter Plate.
5. DNA binding	**STEP:** Bind DNA to silica membrane.	**HOW TO:** Remove and discard the NucleoSpin Plasmid Filter Plate. Open the manifold lid and remove the NucleoSpin Plasmid Binding Plate (white rings) containing the cleared lysates. Insert the Wash Plate on the spacers inside the manifold base, replace the lid on the base and place the NucleoSpin Plasmid Binding Plate on top of the manifold. The vacuum manifold is ready for the next step (**Figure 4**). Apply the vacuum (-0.2 to -0.4 bar, 1 min, flow rate of 1-2 drops per second) until the cleared lysate has drained off, then release the vacuum.

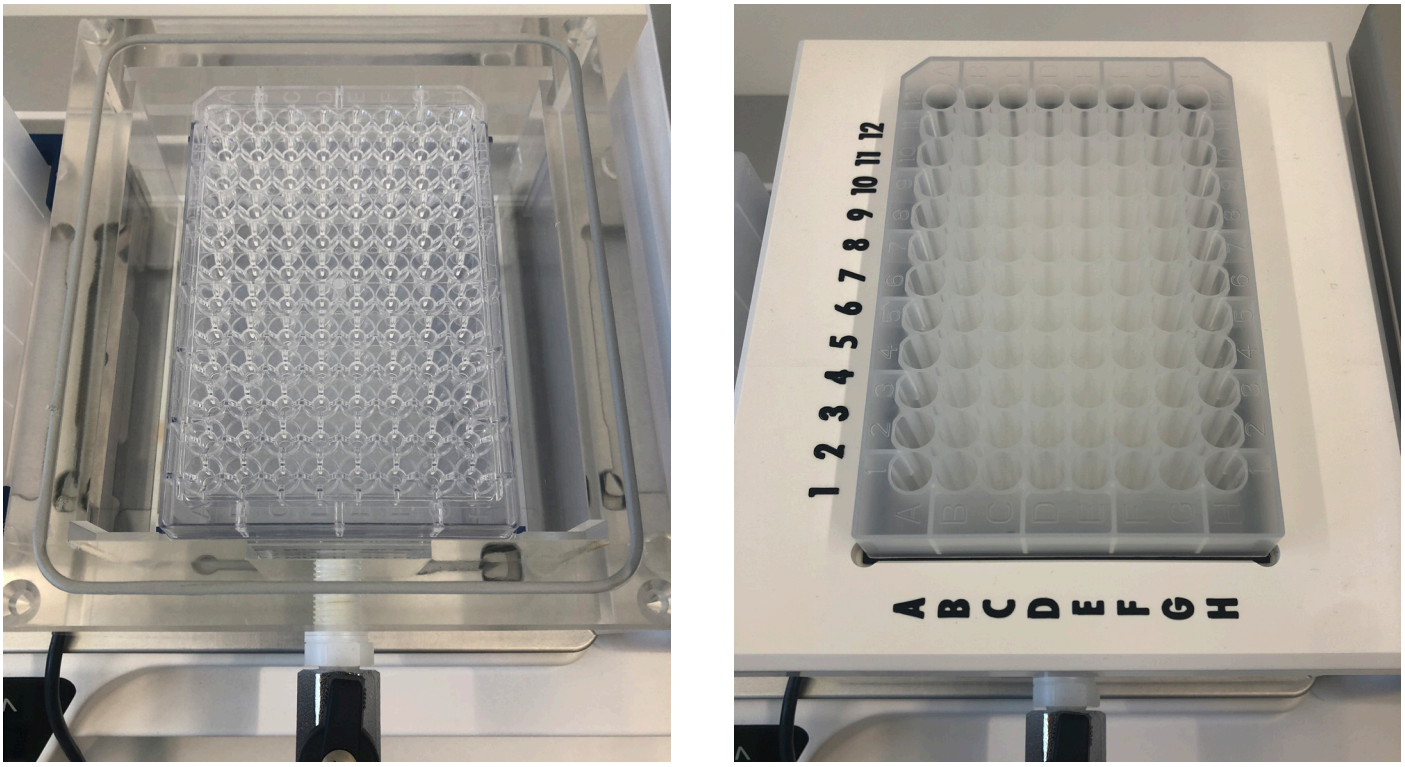


Figure 4: Vacuum manifold set-up for steps 5 and 6. The Wash Plate is inserted on the spacers inside the manifold base (left). The NucleoSpin Plasmid Binding Plate (white rings) with the cleared lysates is placed on top of the manifold (right).

6. Washing and drying

STEP: Wash and dry the silica membrane.

HOW TO: The ASSIST PLUS adds 600 µl Wash Buffer AW to each well. After a 30 second incubation, it informs the user to apply a vacuum (-0.2 to -0.4 bar, 1 min, flow rate of 1-2 drops per second). In the meantime, prepare an 8 row reagent reservoir filled with Wash Buffer A4 (**Figure 5**). Release the vacuum. The ASSIST PLUS transfers 900 µl of Wash Buffer A4 containing ethanol to each well for a second wash step. Using the same conditions as before, apply the vacuum after incubation, release it, and allow the pipette to transfer 900 µl of Wash Buffer A4 to each well for the third wash step. Apply the vacuum after incubation (same settings as before). Remove any residual wash buffer from the NucleoSpin Plasmid Binding Plate and tap the outlets of the plate onto the supplied clean paper sheet. Remove the Wash Plate and the waste container from the manifold base and place the NucleoSpin Binding Plate on top of the manifold. Apply a vacuum (0.4 to -0.6 bar; maintaining a continuous air flow is the most important aspect for this step) for at least 10-15 min to dry the membrane completely and remove any trace of ethanol that may inhibit subsequent enzymatic reactions.

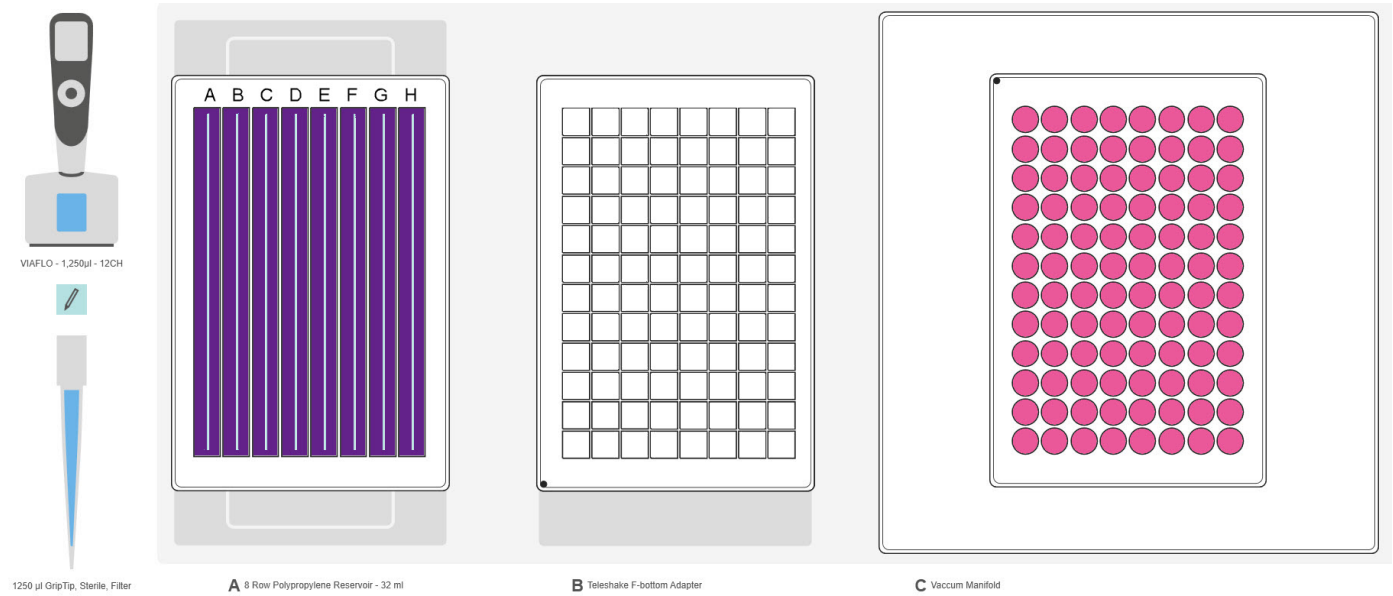


Figure 5: Set-up for the second and third wash steps. The 8 row reagent reservoir in **Position A** is filled with Wash Buffer A4 in rows A to H (purple, 23 ml). In **Position C**, the wells in pink indicate the presence of bacterial DNA bound onto the silica membrane of the NucleoSpin Binding Plate.

Tips:

- Pre-wetting the tips prior to pipetting and having an air gap at the end of the aspiration prevents droplets and dripping when pipetting volatile liquids such as ethanol. Additionally, Low Retention GripTips can be used for these pipetting steps.
- The pipetting speeds have been set up specifically according to the nature of the buffers.

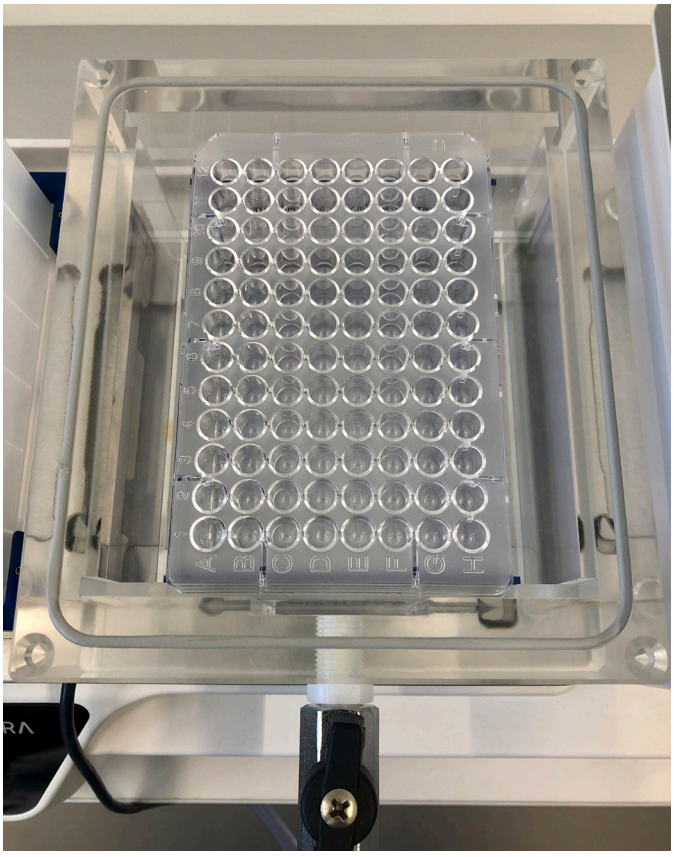
7. Elution

STEP: Eluate the bacterial DNA.

HOW TO: Place the Elution Plate (U-bottom) in the manifold base and the NucleoSpin Plasmid Binding Plate on top of the manifold (**Figure 6**). Place an 8 row reservoir containing Elution Buffer AE in row A on **Position A** (**Figure 7**). The ASSIST PLUS pipetting robot dispenses 150 µl Elution Buffer AE into the Binding Plate. After a 2 minute incubation period, apply a vacuum for 1 minute (0.4 to -0.6 bar), release the vacuum, then remove the Elution Plate containing the eluted DNA and seal it for further storage.

Tip:

- When another DNA concentration is required, simply adapt the elution volume according to MACHEREY NAGEL's recommendations using the VIALAB Software.



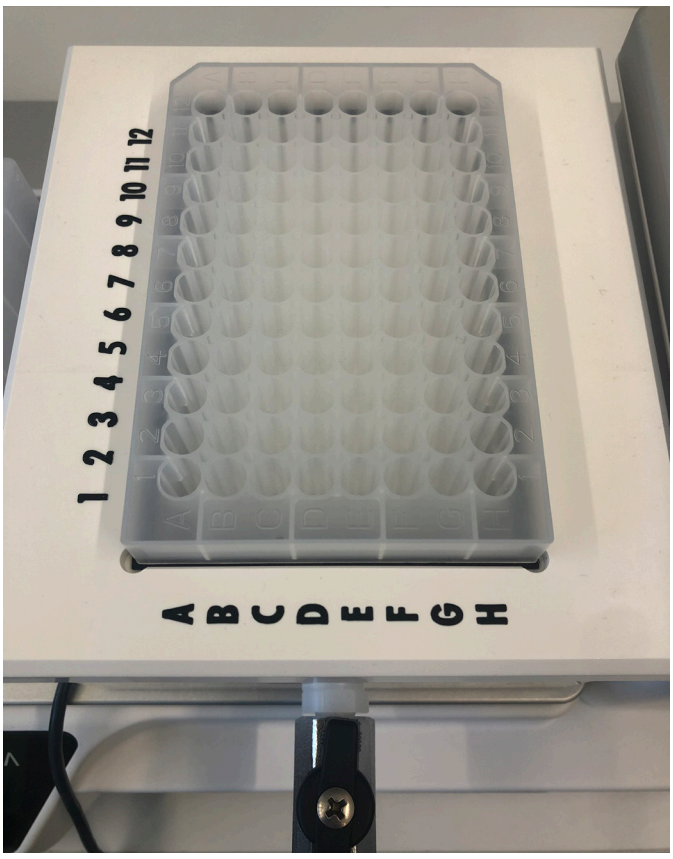


Figure 6: Vacuum manifold set-up for the elution step. The Elution Plate is inserted on the spacers inside the manifold base (left). The NucleoSpin Plasmid Binding Plate (white rings) with the cleared lysates is placed on top of the manifold (right).

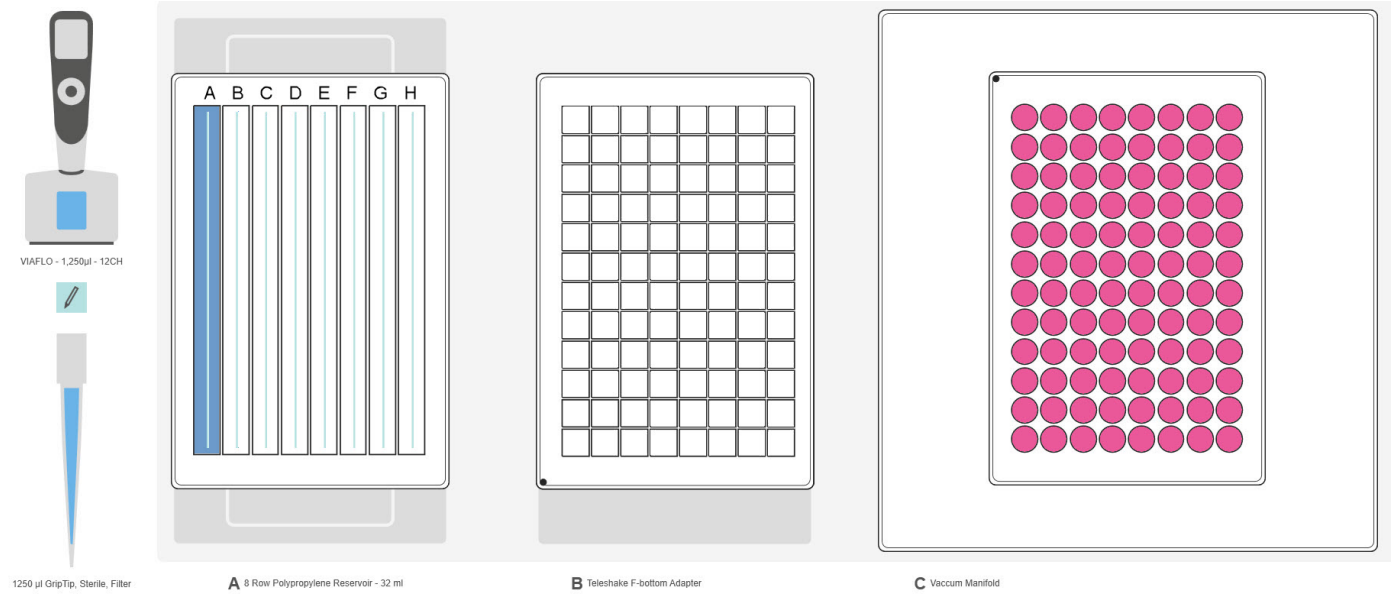


Figure 7: Set-up for the elution step. The 8 row reagent reservoir in **Position A** is filled with Elution Buffer AE in row A (blue, 15 ml). **Position C**: Bacterial DNA bound on the silica membrane of the NucleoSpin Binding Plate (pink).

Remarks

VIALAB software: The VIALAB programs can be easily adapted to your specific labware and protocols, for instance when partial plates are needed, or if lysis of the bacterial cells is performed in tubes.

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

- The MACHEREY-NAGEL NucleoSpin 96 Plasmid kit protocol for plasmid DNA purification can be easily automated with the ASSIST PLUS pipetting robot and a VIAFLO 12 channel 1250 µl electronic pipette. Using the NucleoVac 96 Vacuum Manifold directly on the deck allows for a compact set-up for processing up to 96 samples in one run.
- All the pipetting steps are automated to guarantee maximum reproducibility and consistency of the results, as well as optimal ergonomics for the user to avoid repetitive strain injuries.
- Together with the VIAFLO electronic pipette, the ASSIST PLUS pipetting robot acts as a trusted laboratory assistant and guides the user through the whole protocol, ensuring an error-free workflow.
- The entire purification protocol is included in one single VIALAB program that can be rapidly modified to meet your specific needs.

Materials

Manufacturer	Part Number	Description	Link
INTEGRA Biosciences	4505	ASSIST PLUS base unit	https://www.integra-biosciences.com/en/pipetting-robots/assist-plus
INTEGRA Biosciences	4634	VIAFLO 12 channel 1250 µl electronic pipette	https://www.integra-biosciences.com/en/electronic-pipettes/viaflo
INTEGRA Biosciences	4221	Pipette Communication Module for INTEGRA electronic pipettes	https://www.integra-biosciences.com/en/pipetting-robots/assist-plus#parts-and-numbers
INTEGRA Biosciences	6445	1250 µl Sterile, Filter GripTips	https://www.integra-biosciences.com/en/griptip-selector-guide
INTEGRA Biosciences	6372	8 Row Reagent Reservoirs, Pyramid Bottom, Partitioned (32 ml / row), Pre-Sterilized, 25 units, Polypropylene	https://www.integra-biosciences.com/en/reagent-reservoirs/automation-friendly-reagent-reservoirs
INTEGRA Biosciences	128152	INHECO Teleshake SBS Adapter	https://www.integra-biosciences.com/en/pipetting-robots/assist-plus
INHECO	3800047	Teleshake 230 V	https://www.inheco.com/de/produkte/lab-automation/shaker-lab-automation/shaking-for-labautomation.html
MACHEREY-NAGEL	740488	Culture Plate	https://www.mn-net.com/ch/culture-plates-740488?c=4252
MACHEREY-NAGEL	740625.1 740625.4 740625.24	NucleoSpin 96 Plasmid	https://www.mn-net.com/ch/bioanalytik/kits/plasmid-dna/5167/nucleospin-96-plasmid-96-well-kit-for-plasmid-dna
MACHEREY-NAGEL	740681	NucleoVac 96 Vacuum Manifold	https://www.mn-net.com/nucleovac-96-vacuum-manifold-740681
VACUUBRAND	MZ 2 NT	Vacuum Pump	

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Appendix: Performing the initial deck set-up

Check that the cable of the Teleshake (**Position B**) is not interfering with the movement of the ASSIST PLUS tower. Also make sure that the outlet of the vacuum manifold (**Position C**) is positioned towards the user, so that the tower of the pipetting robot can move freely along the X axis (**Figure 1**).

Place the manifold on the ASSIST PLUS deck next to the waste bin. After adjusting the position of the manifold for the first time, we recommend marking its position on the deck (see example in **Figure 2**). Thereafter, you will simply have to align the vacuum manifold with the marks placed on the ASSIST PLUS.

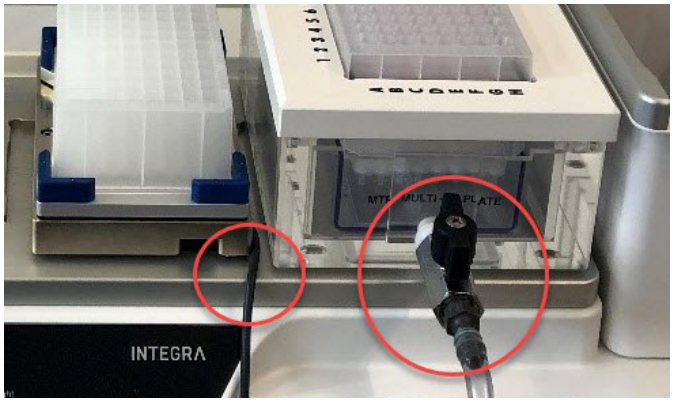

Figure 1: Positioning of the Teleshake cable and the outlet of the vacuum manifold.

FRONT **BACK**

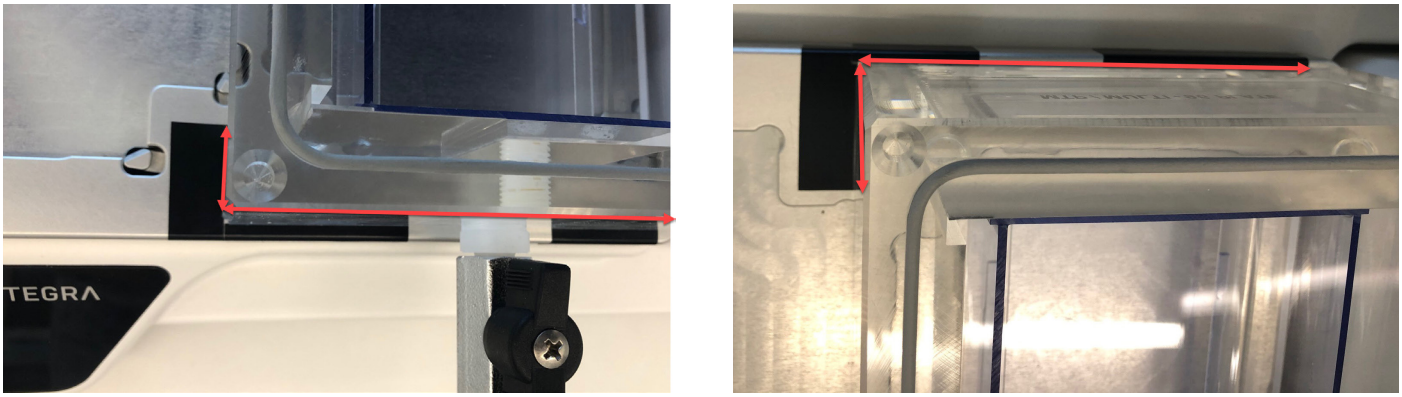

Figure 2: Alignment of the manifold on the deck in Position C. Adding marks on the deck helps to reposition the manifold whenever needed.

To check the position of the well plate on top of the vacuum manifold, manually attach tips to the pipette. Use the touch panel keys to move the pipetting arm of the ASSIST PLUS and to control the tip position. First, select ‘ASSIST PLUS’ under the main menu of the pipette, then ‘VIALAB Programs’ and ‘MN Plasmid’. Go to ‘Height Adjust’, select ‘12 Transfer’ and then choose ‘Height 1/1’ under ‘Target’ using the left arrow. Confirm by pressing the Start Key on the ASSIST PLUS. The ASSIST PLUS moves to the chosen wells. Check the position of the vacuum manifold. The pipette tips should be in the middle of the wells. If necessary, manually adjust the position of the vacuum manifold on the deck. Press the back button on the pipette to exit the Height Adjust menu and discard the tips manually. Continue with the protocol set-up.