APPLICATION NOTE

HIGH-THROUGHPUT PLASMID PURIFICATION WITH TRANSFECTION-GRADE PURITY.

Automated plasmid DNA extraction using the DreamPrep® NAP workstation with additional Te-VacS™ module and MACHEREY-NAGEL's NucleoSpin® 96 Plasmid or NucleoSpin® 96 Plasmid Transfection-grade Kit

TECAN.

INTRODUCTION

Plasmid DNA plays a crucial role in diverse areas of biological research, biotechnology, and therapeutic development, with applications such as cloning, genetic engineering, gene expression, protein production, vaccine development, RNA interference, transfection, and gene delivery studies. Plasmid DNA's ability to carry and express genes of interest makes it a valuable tool for scientists working in the fields ranging from basic molecular biology to advanced medical research applications.

Achieving high-quality plasmid DNA with consistent yields and purity is essential for the success of these applications. The automation of dedicated purification kits on liquid handling workstations can greatly enhance the efficiency, reproducibility, and scalability of plasmid DNA purification or screening processes.

In this application note, we present an automated approach for plasmid DNA purification with both standard and transfection-grade purity using a modified configuration based on Tecan's DreamPrep NAP Workstation for vacuum-based processing in combination with MACHEREY-NAGEL's NucleoSpin 96 Plasmid and NucleoSpin 96 Plasmid Transfection-grade Kits. The workstation's ability to process and handle 96-well plates enables high-throughput processing of samples, making it suitable for large-scale projects and screening applications.

By uniting the capabilities of Tecan's DreamPrep NAP Workstation and MACHEREY-NAGEL's specialized plasmid purification kits, researchers can streamline their plasmid DNA purification workflow, reducing hands-on time and minimizing human errors. The vacuum-based automated process ensures consistent and reproducible results, leading to high yields of pure plasmid DNA. This enables scientists to accelerate their molecular biology research, increase sample throughput, and improve the overall quality of their downstream applications.

MATERIALS AND METHODS

The automated genomic workflow described here was implemented on Tecan's DreamPrep NAP workstation together with an additional Te-VacS module for vacuum processing. The DreamPrep NAP system is based on the Fluent® 480 Automation Workstation and it has been reconfigured for silica membrane-based nucleic acid extraction workflows using the Te-VacS vacuum chamber set-up, in combination with Fluent GX Assurance Software. The DreamPrep NAP is equipped with Air Flexible Channel Arm™, a Robotic Gripper Arm™ and a BioShake™ D30-T elm (QInstruments) for heating and shaking. An integrated Frida Reader™ allows quantification and normalization following nucleic acid extraction. (Figure 1). The tailored protocol allows processing of 1-96 samples per run (variable sample number).

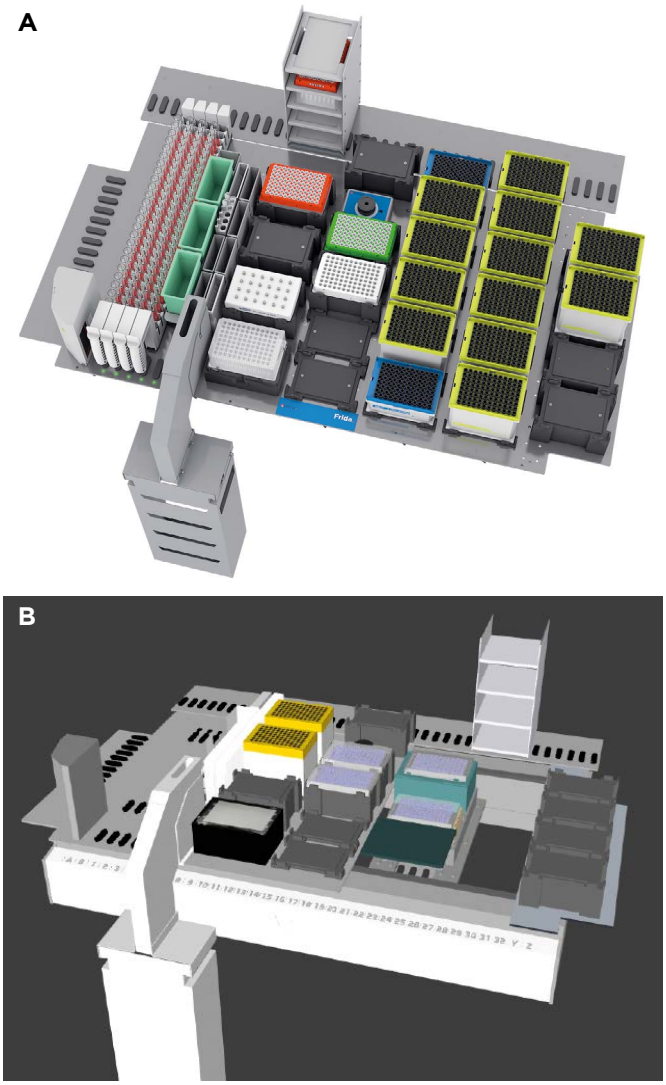


Figure 1: Worktable layout for the standard DreamPrep NAP configuration featuring MACHEREY-NAGEL's worktable (A) and the modified configuration including the Te-VacS vacuum chamber module (B). The integrated Frida Reader module allows for accurate quantification of purified plasmid using UV measurements, with no sample loss during the process.

Plasmid DNA extraction was performed using MACHEREY-NAGEL's NucleoSpin 96 Plasmid or NucleoSpin 96 Plasmid Transfection-grade kits. While both kits utilize silica membrane-based purification technology, the transfection-grade version utilizes specialized technology and buffer chemistry to

additionally remove endotoxins from plasmid DNA for transfection experiments (Table 1 and 2).
The tailored protocols can be used with variable sample numbers, processing 8-96 plasmid purifications per run. The process is fully automated, requiring no manual intervention after cell harvest from up to 5 ml cultures. In short, bacterial pellets were transferred to the DreamPrep Workstation to perform the cell resuspension, alkaline lysis, and neutralization steps. Subsequently, the neutralization reaction was vacuum filtered via the NucleoSpin Filter Plates, directly passing the cleared lysate into the NucleoSpin Plasmid Binding Plate. After binding of plasmid DNA to the silica membrane, contaminants, such as salts, proteins, or endotoxins, were removed by three subsequent washing steps. Highly pure plasmid DNA was finally eluted under low ionic strength conditions in a slightly alkaline elution buffer. For the purification of standard or transfection-grade plasmid DNA, one dedicated script has been developed, allowing for easy kit selection during the startup.

NucleoSpin 96 Plasmid

Technology	Silica membrane technology
Target molecules	Plasmid DNA for standard applications, e.g. genetic engineering / cloning
Sample material	Up to 5 ml bacterial culture
Preparation time	Approx. 70 min / 96 samples
Typical yield	4-30 µg
Endotoxin level	>50 EU/µg DNA

Table 1: NucleoSpin 96 Plasmid – product description.

NucleoSpin 96 Plasmid Transfection-grade

Technology	Silica membrane technology
Target molecules	Plasmid DNA for applications requiring low endotoxin levels, e.g. cell transfections
Sample material	Up to 5 ml bacterial culture
Preparation time	Approx. 70 min / 96 samples
Typical yield	5-20 µg
Endotoxin level	<50 EU/µg DNA

Table 2: NucleoSpin 96 Plasmid Transfection-grade – product description.

RESULTS AND DATA ANALYSIS

Plasmid isolation using NucleoSpin 96 Plasmid

pcDNA3.1 (5.4 kb; high-copy) plasmid DNA was isolated from 1 ml *E. coli* Top10 bacterial cultures using the NucleoSpin 96 Plasmid kit on the DreamPrep NAP workstation. Plasmid DNA was eluted in 150 µl. The eluates were analyzed for yield and purity via UV spectroscopy measurement using the integrated module, Frida Reader. The results below show that the plasmid DNA was isolated with consistent efficiency and purity across the whole 96-purification plate (Figure 2). Purified plasmid DNA can be used for all standard applications such as cloning, sequencing or genetic engineering.

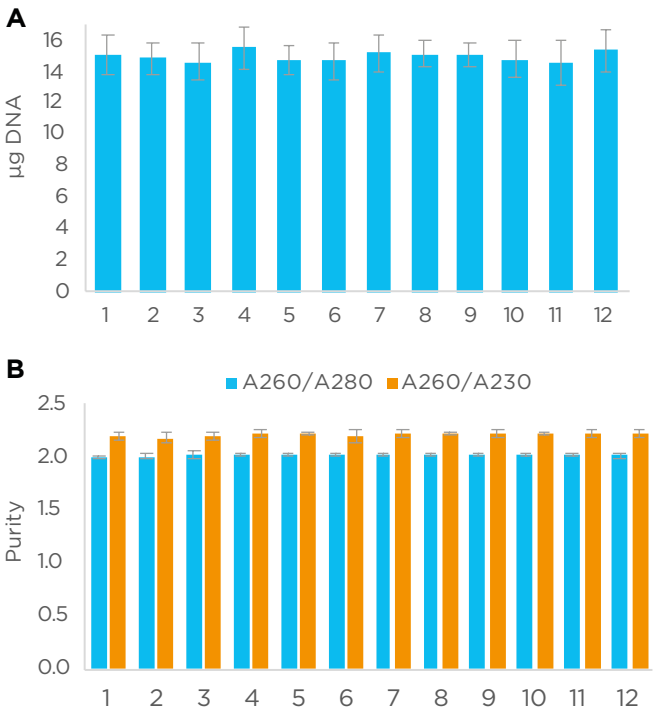


Figure 2: Plasmid DNA was isolated from 1 ml Top10 *E. coli* cultures each using the NucleoSpin 96 Plasmid kit (n=96). The bars represent the average yields (A) and purity (B) in columns 1-12. The overall yield averaged 14.97±1.1 µg with a purity of 2.02±0.02 (A260/A280; blue bar) and 2.21±0.02 (A260/A230; orange bar).

Plasmid isolation using NucleoSpin 96 Plasmid Transfection-grade results in high-quality low-level endotoxin plasmid DNA isolation

Endotoxins, which are lipopolysaccharides present in the cell wall of gram-negative bacteria, are often co-purified during the isolation of plasmid DNA from bacterial cultures. Decreasing endotoxins in plasmid DNA for transfection can greatly enhance the efficiency of transfection in primary cell lines and sensitive cultured cells by minimizing the risk of immune responses and inflammatory reactions caused by endotoxins. These responses can have detrimental effects on cell viability and the uptake of DNA. Through the utilization of the NucleoSpin 96 Plasmid Transfection-grade kit, it is possible to purify plasmid DNA with substantially reduced levels of endotoxins, resulting in a more dependable and effective transfection process and increasing the success rate of DNA delivery into target cells.

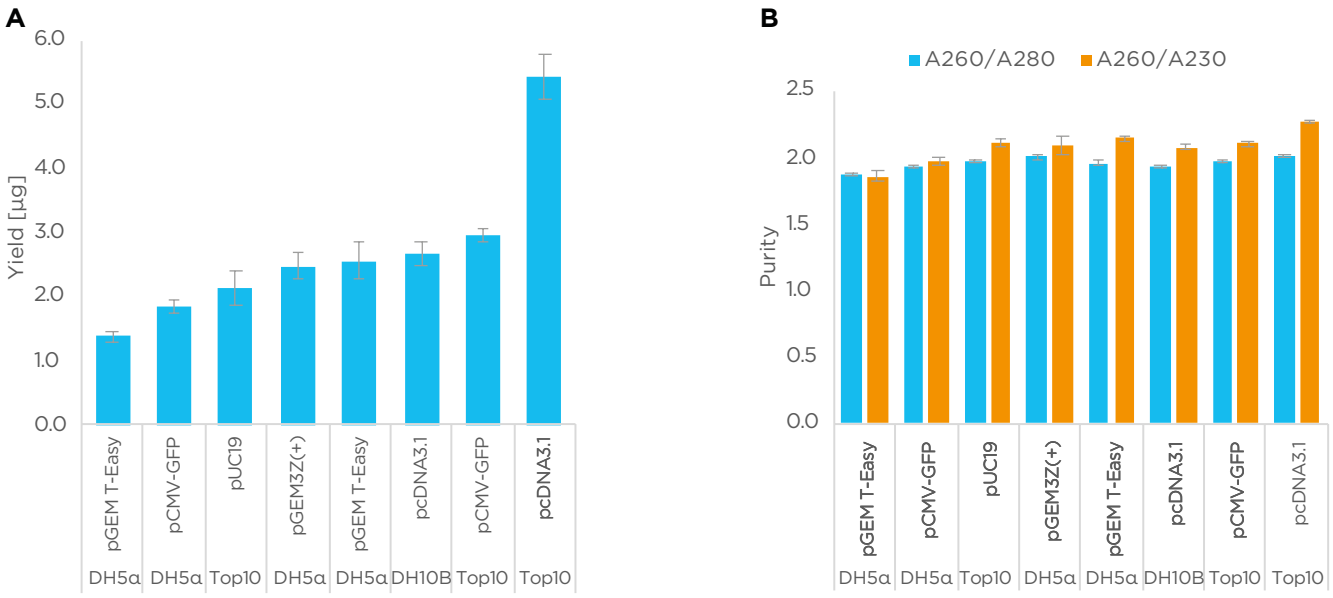


Figure 3: High quality plasmid DNA of eight different plasmid constructs. Yield (A) and purity (B) were determined via UV spectroscopy (n=4 each, randomly arranged on 96-deep well plate).

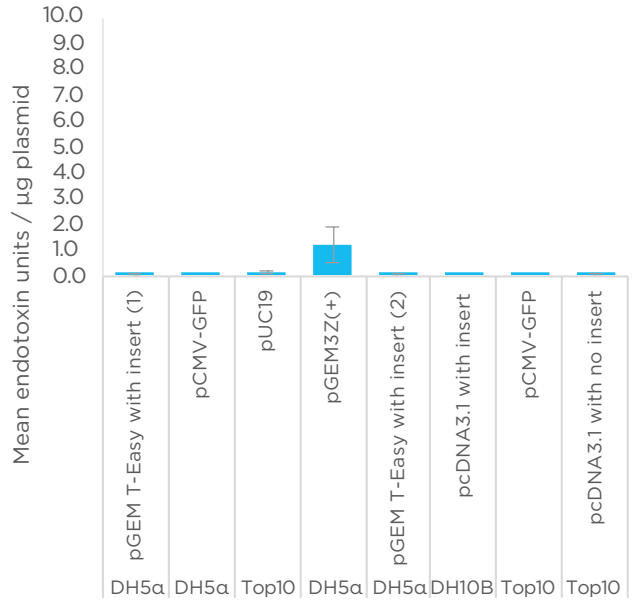


Figure 4: All plasmid constructs showed extremely low endotoxin levels (<5 EU/ µg DNA), making the plasmid DNA highly suitable for transfection experiments (rFC Assay, Lonza).

Different plasmid DNA constructs, known to have different copy-numbers per cell, were isolated from 1 ml *E. coli* Top10 or DH5a bacterial cultures using the NucleoSpin 96 Plasmid Transfection-grade kit (n=4, randomly arranged on 96-well plate). Analyses of eluates show that the plasmid DNA was isolated with both consistent yield and purity across the whole 96-purification plate. Bacterial endotoxin levels were reliably low in all eluates, making the plasmid DNA highly suitable for transfection of sensitive cell lines.

SUMMARY

In their collaborative effort, MACHEREY-NAGEL and Tecan present a customized workflow designed for high-throughput automated plasmid isolation with standard or transfection-grade purity. This innovative approach streamlines the plasmid purification process, enabling researchers to obtain large quantities of high-quality plasmid DNA suitable for transfection experiments. The integration of MACHEREY-NAGEL's purification kits with Tecan's automated liquid handling platforms ensures efficient and reliable purification, enhancing the scalability and reproducibility of plasmid-related experiments in a high-throughput setting.

- Reliable and reproducible plasmid isolations in high-throughput set-ups.
- Flexible sample numbers.
- Standard and transfection-grade plasmid DNA purity.
- Easy and straight-forward workflow: Tecan Fluent workstation can be equipped with additional extension modules such as the Frida Reader for direct plasmid DNA quantity and quality assessment.

LEARN MORE

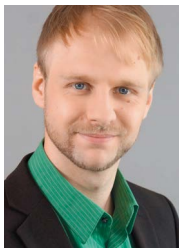
To learn more about Tecan's nucleic acid purification solutions, contact your sales representative or visit **www.tecan.com/NAP**.

To learn more about MACHEREY-NAGEL's plasmid purification solutions, whether from single spin to high throughput solutions or from mini to giga scale, please visit **https://www.mn-net.com/de/bioanalytik/kits/plasmid-dna-information.**

ACKNOWLEDGEMENTS

This protocol was developed by MACHEREY-NAGEL application scientists and is intended for research use only. Users are responsible for determining the suitability of the protocol for their application.

ABOUT THE AUTHORS



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