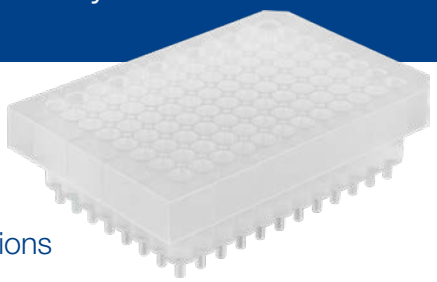


NucleoSpin® 96 Plasmid

Automated high throughput plasmid DNA purification using silica membrane-based kits with Eppendorf's epMotion® liquid handling workstations

Application benefits

Elevate your nucleic acid purification processes using MACHEREY-NAGEL's DNA/RNA purification kits in conjunction with Eppendorf's epMotion® liquid handling systems:

- Flexible sample numbers: Process up to 96 plasmid purifications in parallel.
- Attain consistent recovery of plasmid DNA, ensuring reliable reproducibility in both yield and purity.
- Optimize speed while minimizing plastic consumption for maximum efficiency.
- If you have any questions about MACHEREY-NAGEL's reagents for automation, scripting support, or automation services, feel free to contact us at: automation-bio@mn-net.com for customized assistance.

Keywords

plasmid DNA, nucleic acid extraction, automated plasmid DNA purification, *E.coli* culture, liquid handling system, miniprep, high-purity, high-yield

Introduction

Cloning, genetic engineering, gene expression, protein production, vaccine development, RNA interference, transfection, and gene delivery studies represent a subset of the diverse applications underscoring the significance of plasmids across various domains of biological research, biotechnology, and therapeutic advancement. The inherent capacity of plasmid DNA to transport and express genes of interest positions it as an invaluable tool for scientists engaged in pursuits ranging from fundamental molecular biology investigations to sophisticated medical applications.

The attainment of high-quality plasmid DNA characterized by consistent yields and purity is imperative for the successful execution of these applications. The incorporation of automated liquid handling workstations and specialized purification kits emerges as a pivotal strategy capable of significantly elevating the efficiency, reproducibility, and scalability of plasmid DNA purification and screening processes.

This collaborative application note introduces an automated methodology for plasmid DNA purification. Leveraging Eppendorf's epMotion® in conjunction with MACHEREY-NAGEL's NucleoSpin® 96 Plasmid purification kit, this approach capitalizes on the workstation's capability to process and manage 96-well plates. This feature facilitates high-throughput processing of samples, rendering it particularly suitable for expansive projects and screening applications.

NucleoSpin® 96 Plasmid	
Technology	Silica membrane technology
Sample material	Up to 5 mL bacterial culture
Target molecules	Plasmid DNA for standard applications, e.g. genetic engineering, molecular cloning, transformation or transfection
Typical yield	4–6 µg/mL *E.coli* culture (depends on the efficiency of plasmid propagation, plasmid copy number, and bacterial cell culture density)
Endotoxin level	>50 EU/µg DNA
Preparation time	Approx. 70 min / 96 samples

epMotion® 5057vt	
Technology	Automated liquid handling platform equipped with interchangeable pipetting tools, gripper, heater-shaker and integrated vacuum chamber (further modules are available for different applications).
Sample numbers	1 – 96 samples
Deck position	Configurable platform with 12 SLAS-/ANSI slots + trash for liquid and solid waste
Pipetting volume	0.2–1000.0 µL

Figure 1:
The Eppendorf epMotion® 5075vt offers versatility through the incorporation of various pipetting modules, a gripper, heat shaker, and a vacuum chamber. This configuration allows for the processing of both silica membrane-based NucleoSpin® 8/96 and magnetic bead-based NucleoMag® DNA/RNA extraction kits.

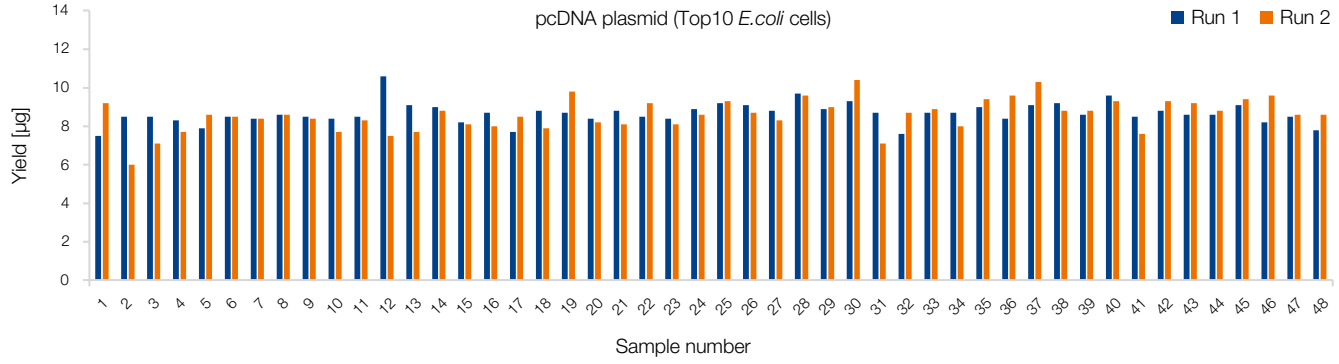
Material and Methods

The extraction of plasmid DNA was carried out utilizing MACHEREY-NAGEL's NucleoSpin® 96 Plasmid system on an Eppendorf epMotion® 5075vt platform. The biologically-verified protocol allows for flexibility in sample numbers, accommodating 8-96 plasmid purifications per run. The entire process is automated, eliminating the need for manual intervention post-cell harvest from cultures of up to 5 ml. Briefly, bacterial pellets were transferred to the Eppendorf epMotion® for cell resuspension, alkaline lysis, and neutralization steps. Following this, the neutralization reaction underwent vacuum filtration through NucleoSpin® 96 Filter Plates, efficiently directing the clarified lysate into the NucleoSpin® 96 Plasmid Binding Plate. After binding plasmid DNA to the silica membrane, contaminants such as salts, proteins, or endotoxins were removed through three consecutive washing steps. The highly purified plasmid DNA was ultimately eluted under low ionic strength conditions using a slightly alkaline elution buffer.

All pipetting and vacuum filtration steps were carried out by the Eppendorf epMotion® 5075vt.

Application Data

A



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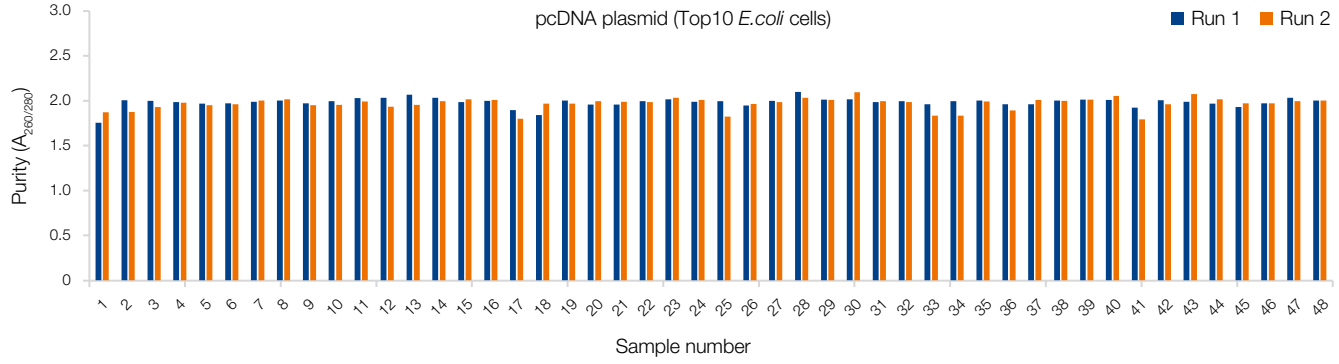

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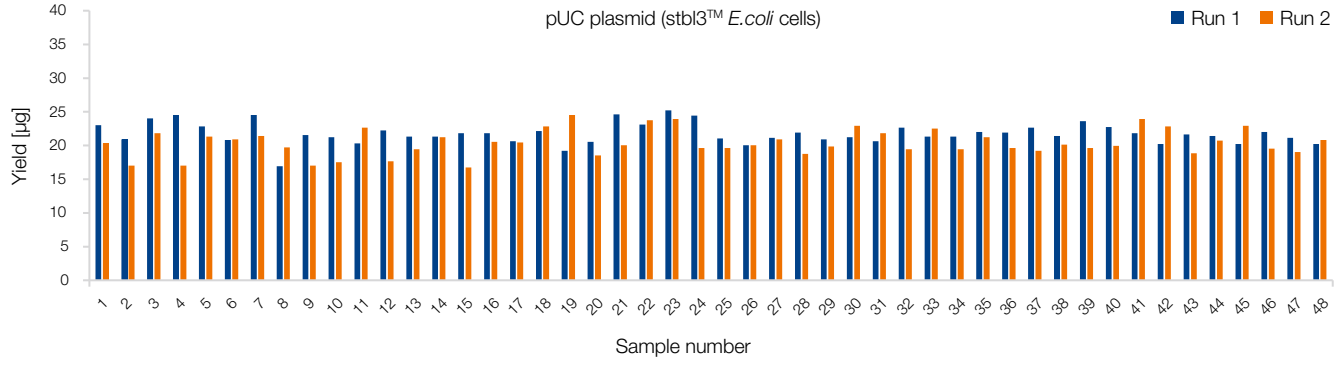

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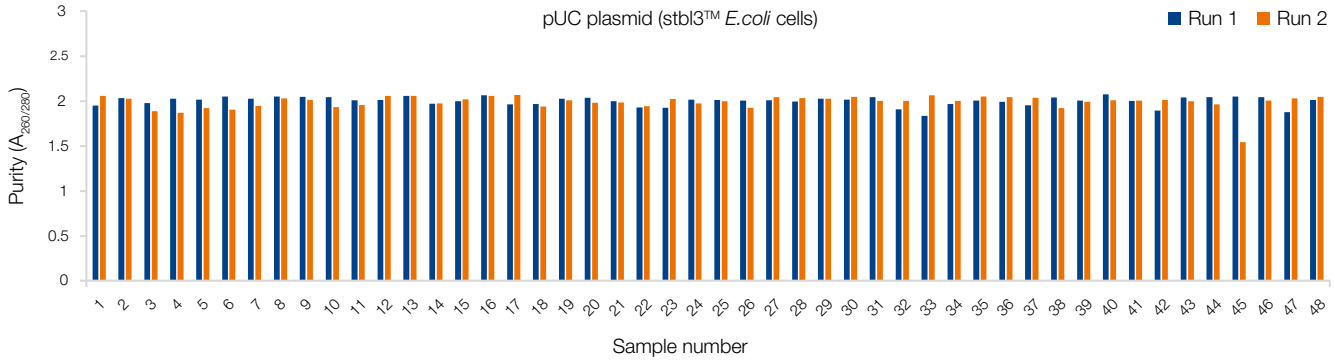

Plasmid DNA Purification Efficiency and Purity Analysis

The plasmid DNA purification process was conducted using the MACHEREY-NAGEL NucleoSpin® 96 Plasmid kit on the Eppendorf epMotion® 5075vt liquid handling platform. Two distinct strains were utilized, one carrying the pcDNA plasmid (Top10 *E.coli* cells, graphics A and B) and the other carrying the pUC plasmid (Stbl3™ *E.coli* cells, graphics C and D). Yield and purity assessment were performed via UV spectrometry, demonstrating exceptional reproducibility and repeatability within individual runs (samples 1-48) and across multiple runs (run 1, blue bar; run 2, orange bar). The average yield for the pcDNA plasmid was 21.06 ± 1.86 µg, while the pUC plasmid yielded 8.63 ± 0.70 µg. The overall purity of the purified pcDNA plasmids exhibited an average $A_{260/280}$ ratio of 1.99 ± 0.07 and an $A_{260/230}$ ratio of 2.26 ± 0.17. Similarly, for the pUC plasmid, the overall purity averaged an $A_{260/280}$ ratio of 1.97 ± 0.06 and an AA260/230 ratio of 2.20 ± 0.08. While the final yield of purified plasmid DNA is influenced by factors such as the starting bacterial culture and specific characteristics of the plasmid (including its size and propagation efficiency), the findings demonstrate that plasmid DNA purification using the vacuum-based NucleoSpin 96 Plasmid kit consistently yields reliable results, showing remarkable repeatability and reproducibility irrespective of the plasmid construct or bacterial strain.

Ordering information

Product	Specifications	Pack of	REF
NucleoSpin® 96 Plasmid	Silica membrane-based kit for the isolation of plasmid DNA from bacterial cultures, including NucleoSpin® Plasmid Binding Plates, NucleoSpin® Filter Plate, Culture plates, gas permeable filis, elution plates, wash plates, buffers, and RNase A (lyophilized)	1 × 96 preps 4 × 96 preps 24 × 96 preps	740625.1 740625.4 740625.24
Eppendorf epMotion® 5075vt*	Automated liquid handling platform including MultiCon PC, a completely self-contained housing, vacuum system with accessories, gripper, ThermoMixer®, epBlue software, keyboard, mouse, system for solid and liquid waste, 100–240 V/50–60 Hz	1-96 samples	5075 000 044*

NucleoSpin® is a registered trademark of MACHEREY NAGEL (contact: automation-bio@mn-net.com);

*For more detailed information, please visit *www.eppendorf.com*. To contact Eppendorf SE, please email eppendorf@eppendorf.com or visit *www.eppendorf.com/contact*.