

Fast and efficient PCR clean-up

Reliable purification of PCR products with high yield and purity using the MACHEREY-NAGEL NucleoSpin[®] 96 PCR Clean-up kit on a Freedom EVO[®] platform

Introduction

PCR has become a standard molecular biology tool for biological and medical research laboratories, and is used for DNA cloning, sequencing and genetic testing. For most applications, PCR products need to be purified, and this is advantageous to perform in a high throughput format. Tecan and MACHEREY-NAGEL have joined forces to provide a flexible automated solution for the purification of PCR products for research use only, not for use in clinical diagnostics.

MACHEREY-NAGEL's NucleoSpin 96 PCR Clean-up kit for fast purification of PCR products is suitable for a broad range of downstream applications, including restriction digest, cloning and sequencing. The purification method is based on vacuum filtration – using silica membranes in combination with suitable binding, wash and elution buffers – and can be fully automated on the Freedom EVO platform. The system can be set up in a matter of minutes, offering long walkaway times and relieving staff from tedious, repetitive tasks.

Common risks – such as cross-contamination, carry-over of chemicals and solvents, and manual handling errors – are minimized, while improving reproducibility, and process security can be further enhanced by full sample tracking.

Fully automating the PCR product purification process on a Freedom EVO workstation streamlines laboratory workflows, enabling rapid, reliable purification of 96 samples in 50 min. The purity of the purified PCR products is demonstrated by an average $A_{260/280}$ ratio between 1.7 and 1.8, and recoveries are excellent, with yields in excess of 78 %.

Materials and methods

Equipment

The Freedom EVO liquid handling can be equipped with a 2-, 4- or 8-channel Liquid Handling (LiHa) Arm using disposable tip adapters and the low level disposable tip ejection option to reduce cross-contamination (Figure 1 and Table 1). A Te-VacS™ vacuum module is accessed using a Robotic Manipulator (RoMa) Arm, and can accommodate either 96-well binding plates or 8-well binding strips.

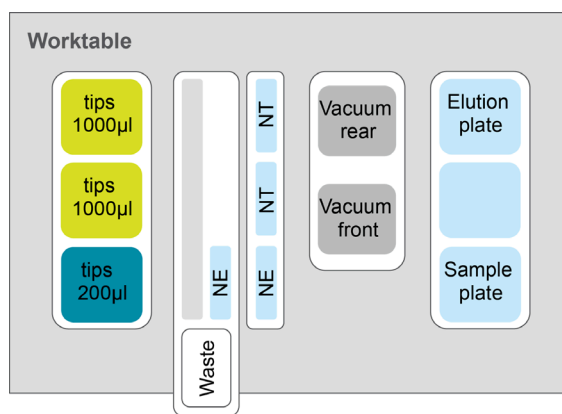


Figure 1 Freedom EVO worktable layout for purification of PCR products

Sample numbers	Up to 96 samples, in multiples of 8 or 96
Batch time	50 min for 96 samples
Tecan equipment	<ul style="list-style-type: none"> Freedom EVO 100 platform, 8-channel Liquid Handling Arm configured for disposable tips, 1,000 µl syringes, Robotic Manipulator Arm, stainless steel deck and safety panel set Microplate, trough (100 ml) and disposable tip carriers Wash station with waste container 200 and 1,000 µl disposable tips (filtered) Freedom EVOware® Standard software package
MACHEREY-NAGEL equipment	<ul style="list-style-type: none"> NucleoSpin 96 PCR Clean-up kit Column holder A (required for 8-well strips only)

Table 1 Overview of equipment for medium and high throughput PCR product purification

Automated workflow

Up to 100 µl of each PCR product is placed onto the platform and purified without any user intervention. The fully automated purification procedure includes binding of PCR products to silica membranes, stringent wash steps and, finally, the elution of the purified PCR products.

The configuration and scripting of the Freedom EVO workstation has been optimized to minimize the risk of cross-contamination and maximize the yield and quality of the PCR products.

Results

Automation of the NucleoSpin 96 PCR Clean-up kit on the Freedom EVO sample preparation workstation enables fast and reliable purification of PCR products of variable length, offering high recovery rates and excellent purity.

Purity

Automated purification yielded high quality PCR products, with an average $A_{260/280}$ ratio of between 1.7 and 1.9 (Figure 2).

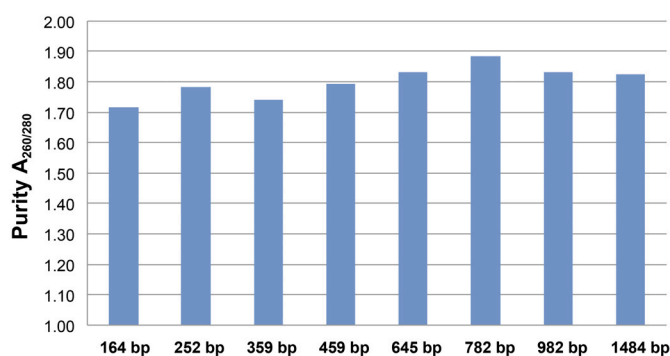


Figure 2 Purified PCR products demonstrated $A_{260/280}$ ratios of 1.7 or above. Each bar represents the average $A_{260/280}$ ratio from eight samples

The purity of the PCR products was confirmed by gel electrophoresis (Figures 3 and 4).

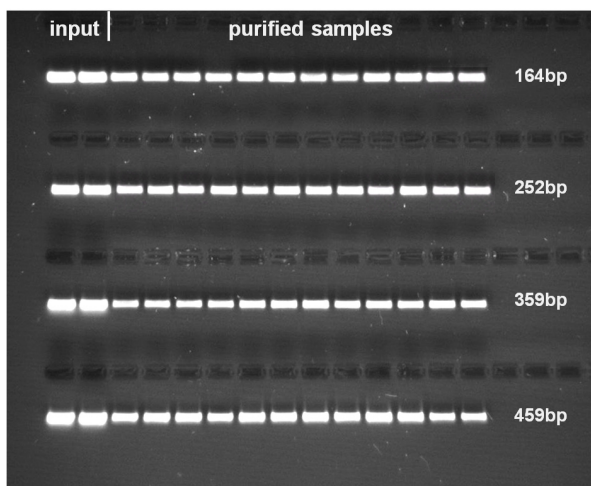


Figure 3 High purity of PCR products, as demonstrated by gel electrophoresis for small PCR products between 150 bp and 500 bp

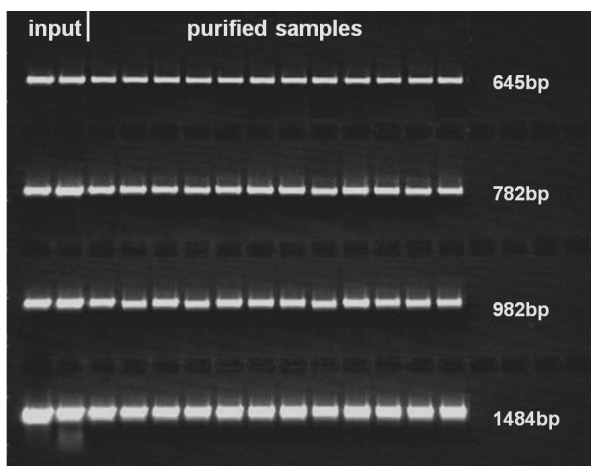


Figure 4 High purity of PCR products, as demonstrated by gel electrophoresis for medium size PCR products between 600 bp and 1500 bp

Recovery

The concentration of PCR product was measured before and after purification, and the automated process demonstrated excellent recovery rates of around 80 to 90 % (Figure 5).

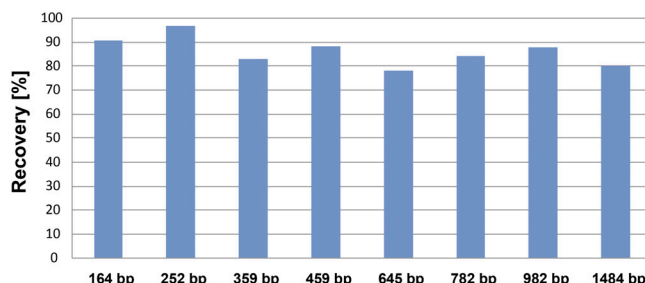


Figure 5 Purified PCR products showed recoveries in excess of 75 %. Each bar represents the average recovery from eight samples.

Conclusion

Automation of the NucleoSpin 96 PCR Clean-up kit on a Freedom EVO sample preparation workstation enables fast purification of PCR products in a true walkaway manner. For maximum flexibility, or to meet changing laboratory needs, the Freedom EVO workstation can be equipped with a variety of modules, including an absorbance reader, storage modules and cooling devices. For user-friendly, step-by-step guidance through the instrument set-up process, the PCR clean-up protocol can be embedded into the visual workflow of the Nucleic Acid Purification Wizard (Figure 6).

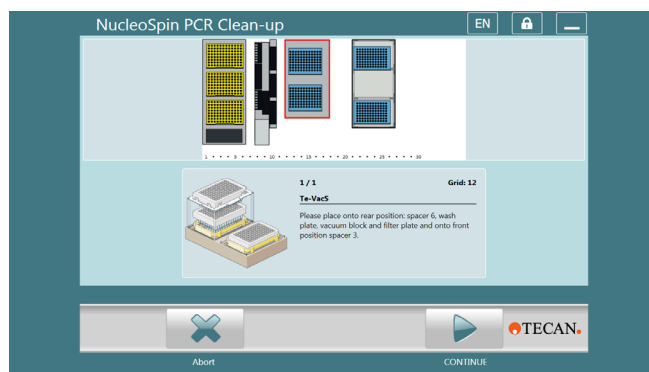


Figure 6 The Nucleic Acid Purification Wizard guides the user through the set-up process

Talk to your local Tecan representative to learn how to customize the Freedom EVO workstation to your specific laboratory requirements.

Acknowledgements

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Further application notes

The current list of application notes can be found at www.tecan.com/machereynagel

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