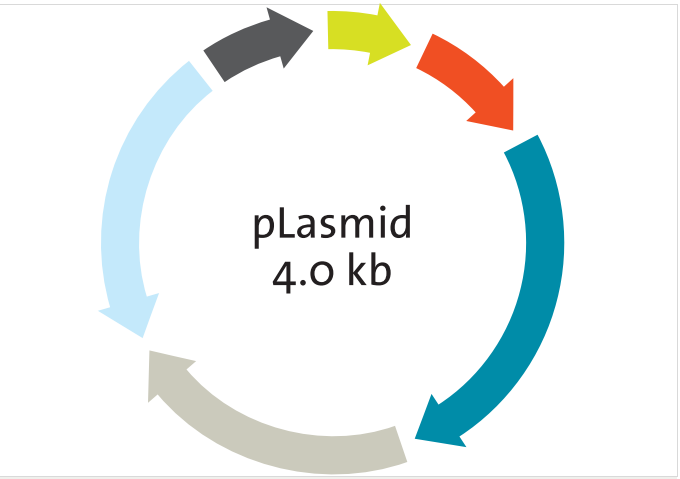

High purity plasmids

Reliable extraction of exceptional purity plasmid DNA using the NucleoSpin® 96 Plasmid kit on a on a Freedom EVO® platform

Introduction

For over 20 years, the extraction of plasmid DNA has been part of the daily routine of almost every molecular biology laboratory. Plasmids are used to clone and modify genes of interest, as DNA cloned into suitable plasmids can easily be analyzed by restriction digests and sequencing. A more recent application, involving high throughput plasmid purification, is the production of plasmids for RNA interference (RNAi) applications. However, purification of plasmid DNA remains a time-consuming step in genetic analysis, creating a need for automated high throughput extraction techniques.

Tecan and MACHEREY-NAGEL have joined forces to provide a flexible, fully automated solution for the purification of plasmid DNA using the Freedom EVO workstation, streamlining laboratory workflows and providing reliable, fast extraction of high purity plasmid DNA.

MACHEREY-NAGEL's NucleoSpin® 96 Plasmid kit offers fast extraction of high purity plasmid DNA, and is suitable for a full range of downstream applications, such as sequencing. The extraction method is based on a modified alkaline lysis procedure in combination with silica membranes, and the kit is available in 96-well plate or 8-well strip formats.

Vacuum-based extraction of either format can be fully automated on the Freedom EVO platform, and can be set up in a matter of minutes. Considerable walkaway time is gained, relieving staff from tedious repetitive tasks and allowing them to perform more highly skilled operations. The procedure reduces the risks commonly associated with automation, such as sample cross-contamination and carry-over of chemicals, while avoiding manual errors and achieving maximum reproducibility. In addition, full sample tracking further improves overall process security.

The outstanding purity of the extracted DNA is demonstrated by an average $A_{260/280}$ ratio of 1.9, as well as excellent sequencing and restriction digest performance. A 1.5 ml

aliquot of an *E. coli* culture (DH5a culture grown in LB medium overnight) typically yields between 3 and 10 µg of DNA, strongly depending on the purified plasmid. Full automation of the plasmid purification process on a Freedom EVO workstation streamlines laboratory workflows and provides reliable and fast extraction of highly pure plasmid DNA.

Material and methods

Equipment

The Freedom EVO liquid handling workstation can be equipped with a 2-, 4- or 8-channel Liquid Handling (LiHa) Arm, with disposable tip adaptors and low level disposable tip ejection options to reduce cross-contamination. A Robotic Manipulator (RoMa) Arm configures the Te-VacS™ vacuum station, which can accommodate either 96-well filter plates or the MACHEREY-NAGEL 8-well filter strips. The system also includes a Te-Shake™ module for fast, optimal mixing of samples and buffers (Figure 1).

	High and medium throughput
Sample numbers	Up to 96 samples, in multiples of 8 or 96
Batch time	1 h 25 mins for 96 samples
Tecan Equipment	• Freedom EVO 100 platform, 8-channel Liquid Handling Arm configured for disposable tips, 1000 µl syringes, Robotic Manipulator Arm, stainless steel deck and safety panel set • Te-VacS • Te-Shake • Microplate, trough, tube and disposable tip carriers • Wash station with waste disposal • Disposable tips (filtered) 1000 µl and 100 ml troughs • Freedom EVOware® Standard software package
MACHERY-NAGEL Equipment	• NucleoSpin 96 Plasmid kit • Square-well blocks • Column holder A (required for 8-well strips only)

Table 1 Overview of equipment required for plasmid extraction

Automated workflow

Configuration and scripting of the Freedom EVO workstation have been optimized to minimize the risk of cross-contamination and maximize the DNA yield and quality of nucleic acids.

After overnight incubation of bacterial cultures in a 96-well plate, the cultures are centrifuged and the supernatant discarded. The bacterial pellets are then placed onto the Freedom EVO workstation and the plasmid DNA extraction is completed without further user intervention. The fully automated plasmid purification procedure includes lysate filtration into the NucleoSpin Plasmid Filter Plate, binding of the DNA to the NucleoSpin Plasmid Binding Plate, stringent wash steps and final elution of the purified DNA in 75 to 150 µl of elution buffer, depending on the subsequent use of the DNA.

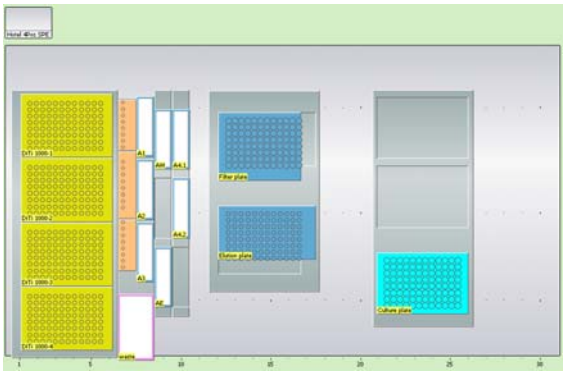

Figure 1 Freedom EVO worktable for plasmid extraction with the Te-VacS and Te-Shake modules and use of the Robotic Manipulator Arm and the Liquid Handling Arm.

Results

Automation of the NucleoSpin 96 Plasmid kit on the Freedom EVO sample preparation workstation allows fast, convenient and reliable purification of plasmid DNA. The plasmid yield achieved by the automated method is equivalent to that of the manual method (Figure 3), with significantly improved DNA purity (Figure 2). Moreover, the DNA purity obtained with the automated method is highly consistent within each set of DNA inserts and all the constructs tested. The complete automated extraction of 96 samples, starting from the bacterial pellet, takes 1h 25 mins.

Purity

The purity of plasmid DNA extracted using MACHEREY-NAGEL's NucleoSpin 96 Plasmid kit is of outstanding quality. With $A_{260/280}$ ratios averaging 1.9, the eluted DNA is of excellent purity and is therefore suitable for a full range of downstream applications, including sequencing, PCR and restriction digests.

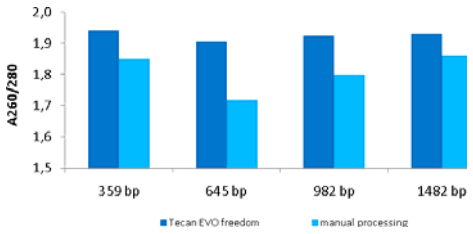

Figure 2. Plasmid purity – comparison of manual and automated processing. Each bar represents the average $A_{260/280}$ ratio from 24 extractions of plasmid DNA from 1.5 ml overnight cultures, processed either manually or using the automated method. Insert lengths of the pGEM plasmids are as indicated

Yield and reliability

As shown in Figure 3, the yield of plasmid DNA from a 1.5 ml overnight bacterial culture varies between 3 and 6 µg, depending on the plasmid construct, with the automated and manual processes giving equivalent yields. Reproducibility and intra assay variance was tested in following experiment. Plasmid DNA isolated from 96 aliquots of one large bacterial culture gave a very consistent yield of DNA, with a CV of 12 % (Figure 4).

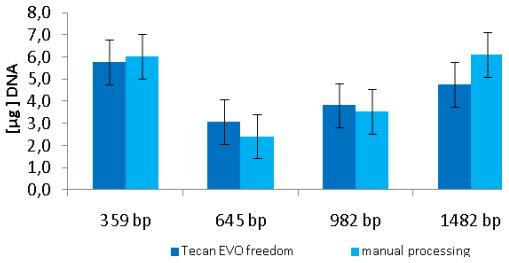

Figure 3 Plasmid yields obtained by manual and automated DNA isolation. Each bar represents the average yield (µg) from 24 extractions of plasmid DNA from 1.5 ml overnight cultures. DNA yields of four pGEM plasmids with different inserts are shown.

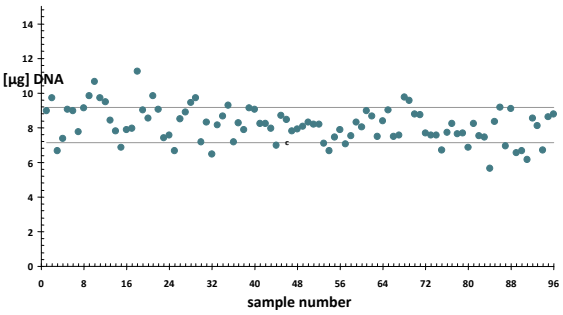

Figure 4 Reliability of plasmid DNA extraction. Plasmid DNA from 96 identical overnight bacterial cultures was isolated using the automated method. The mean DNA yield was 8.15 µg, with a CV of 12 %.

Downstream applications

The purified DNA is suitable for a broad range of downstream applications, including restriction digestion and sequencing. This is demonstrated in Figure 5, which shows a restriction digestion of two plasmids with EcoRI enzyme. Without digestion, the plasmid is visible as a major band of the ccc form, showing a structural integrity of > 90 %. Other configurations, such as oc, are also visible as faint bands and these distinct bands indicate the absence of DNase activity. After digestion, two DNA fragments of the expected size are obtained. The DNA digest was successful in all cases, highlighting the excellent quality of the plasmids and the absence of potential inhibitors or contaminants.

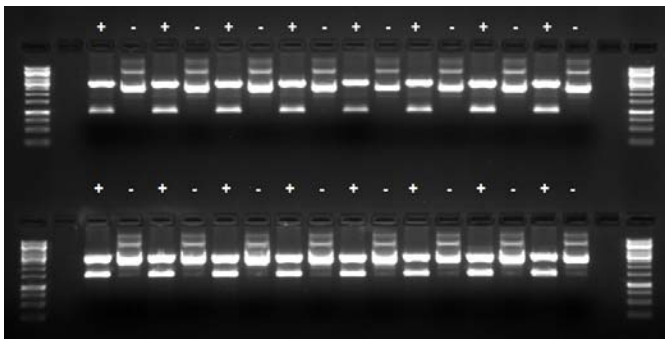

Figure 5 Restriction digestion with EcoRI. + indicates a restriction digest, – indicates no restriction digest. 10 µl of two different plasmids, with inserts of 359 bp (top) and 645 bp (bottom), were subjected to restriction digest. Samples were incubated for 2 h at 37 °C with EcoRI restriction enzyme. After digestion, samples were analyzed by 1 % agarose gel electrophoresis. Marker Lamda Hind III (Frementas).

Figure 6 shows the sequencing profile of a plasmid purified using the automated method. Very sharp peaks with very low noise are observed and can be easily analyzed, demonstrating the high purity of the isolated plasmid DNA. Long read lengths with high quality scores were obtained.

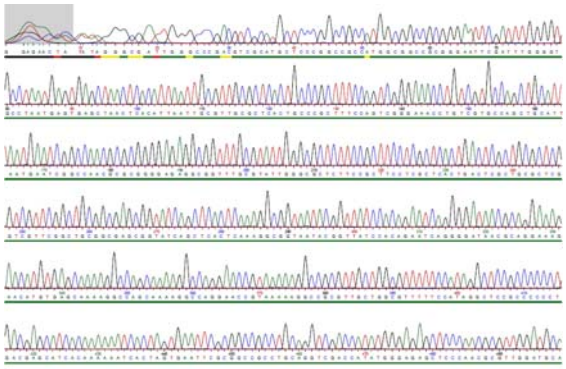

*Figure 6 Sequencing profile of a plasmid purified using the automated method. To demonstrate the quality of the purified DNA for sequencing, DNA samples were sequenced using the Applied Biosystems BigDye® chemistry. The DNA sequence was determined on an Applied Biosystems 3730*XL *capillary sequencer.*

Conclusion

Automation of the NucleoSpin 96 Plasmid kit on the Freedom EVO sample preparation workstation allows fast, reliable extraction of plasmid DNA in a true walkaway manner, consistently generating high quality DNA. For maximum flexibility, or to meet changing laboratory needs, the Freedom EVO sample preparation workstation can be equipped with a number of extension modules, including an absorbance reader, storage modules and cooling devices.

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

Acknowledgements

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Further Application Notes

A full list is available at www.tecan.com/machereynagel

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