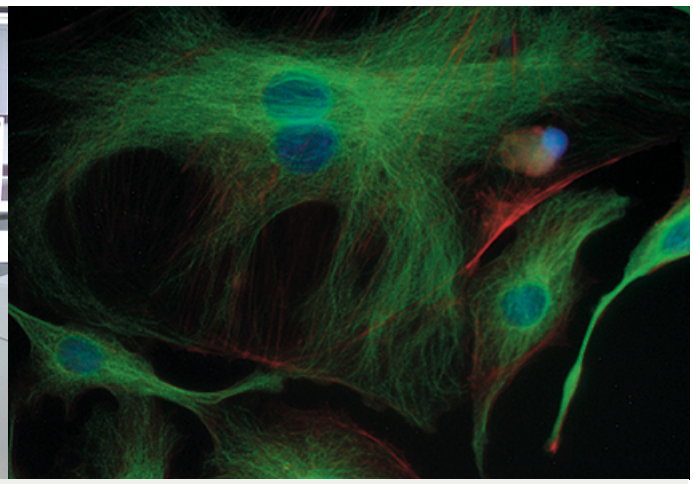

Pure gDNA from tissue

Reliable extraction of gDNA from cells and tissue samples with high yield and purity using the NucleoSpin® 96 Tissue kit on a Freedom EVO® platform

Introduction

Purification of large numbers of cell and tissue samples represents a serious bottleneck in sample processing for PCR based genotyping methods or large scale breeding projects and screening tasks in cancer research. Furthermore, reliability process control and the avoidance of cross-contamination are extremely important for the extraction of gDNA.

Tecan and MACHEREY-NAGEL have combined forces to provide a flexible automated solution for the purification of gDNA from cell and tissue samples for research use only, not for use in clinical diagnostic.
MACHEREY-NAGEL's NucleoSpin® 96 Tissue kit for fast purification of highly pure genomic DNA is suitable for a broad range of downstream applications, such as PCR and real-time PCR. 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, gaining considerable walkaway time and relieving staff of tedious repetitive jobs, freeing them to perform more highly skilled tasks. This automated solution reduces common risks such as cross-contamination between samples and carry-over of chemicals and solvents, while reducing manual errors and maximizing reproducibility. In addition, full sample tracking further improves overall process security.
The high purity of the purified DNA is demonstrated by an average A260/280 ratio of 1.9, in addition to excellent real-time PCR performance. DNA yields of up to 10 µg gDNA (average 6.5 µg) are obtained from 3 mm mouse tail end clippings. Full automation of the gDNA purification process on a Freedom EVO® workstation streamlines laboratory workflows and provides reliable, fast extraction of highly pure gDNA.

Materials 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 adapters and low level disposable tip ejection options to reduce cross-contamination. A Robotic Manipulator (RoMa) Arm configures the Te-VacS™ vacuum module, which can accommodate either MACHEREY-NAGEL's 96-well binding plates or the 8-well binding 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 20 min for 96 samples
Equipment Tecan	• 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-Shake • Microplate, trough, tube and disposable tip carriers • Wash station with waste • Disposable tips (filtered) 1000 µl and 100 ml troughs • Freedom EVOware® Standard software package
Equipment MACHEREY-NAGEL	• NucleoSpin® 96 Tissue kit • Column Holder A (required for 8-well strips only)

Table 1 Overview of equipment for gDNA purification from cells or tissue samples

Automated workflow

Typically, samples of 200 µL lysed tissue are placed onto the platform and the genomic DNA is purified without any user intervention. The fully automated gDNA purification procedure includes lysis of the samples (optionally, e.g. for processing cultured cells), binding of genomic DNA to silica membranes, stringent wash steps and finally the elution of purified gDNA.

The configuration and scripting of the Tecan workstation have been optimized to minimize the risk of cross-contamination and maximize the yield and quality of nucleic acids.

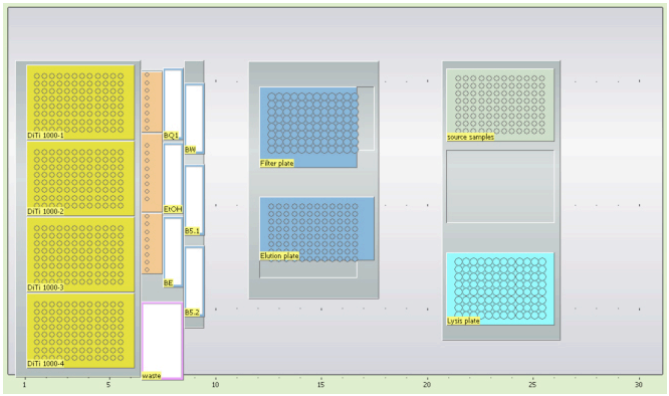

Figure 1 Freedom EVO worktable for gDNA isolation from cells and tissue samples with the Te-VacS and Te-Shake modules and use of the Robotic Manipulator Arm and the Liquid Handling Arm

Results

Automation of the NucleoSpin® 96 Tissue kit on the Tecan Freedom EVO® sample preparation workstation allows fast, convenient and reliable purification of gDNA from a variety of tissue sources (up to 20 mg) or up to 10^6 cultured cells or bacteria.

The automated method produces isolated DNA of excellent purity with average A 260/280 ratios of 1.9 (Figure 2), and the yield is consistently high (Figure 3). Both parameters are comparable between the manual and automated methods. The complete automated extraction of gDNA from 96 lysates from tissue samples takes 1h 20 mins.

DNA Purity

The purity of DNA purified with MACHEREY-NAGEL's NucleoSpin® Tissue kit is excellent. With mouse tail samples showing an average A260/280 ratio of 1.9 (Figure 2), the eluted DNA is highly pure and free of contaminants, allowing for a broad range of downstream applications, such as PCR, real-time PCR or restriction analysis.

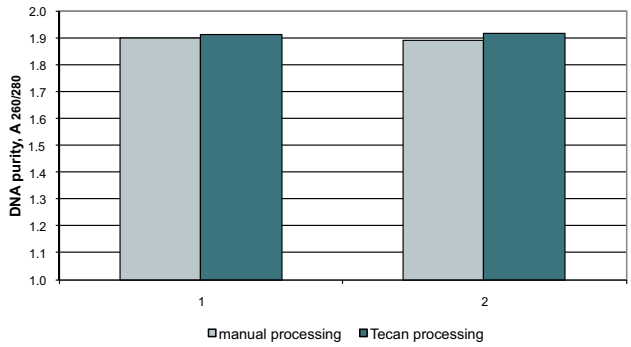

Figure 2 Excellent purity of gDNA purified from mouse tail samples
Each bar represents the average A260/280 ratio from a lysate of 3 mm mouse tail end clipping samples (n=24). For the corresponding DNA yields see Figure 4.

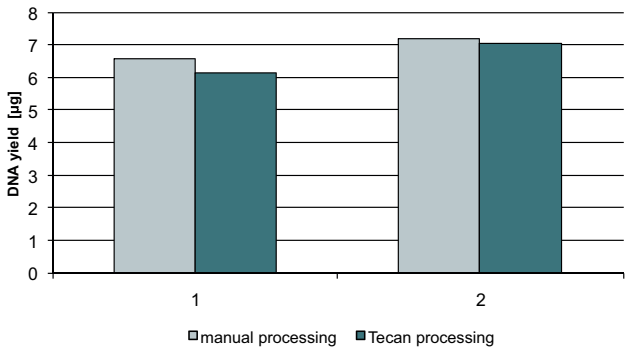

Figure 4 Average yield of gDNA isolated from mouse tail end clippings
Each point represents the average from 3 mm mouse tail end clipping samples (n=24).The yield between the automated and manual method is comparable.

Yield and Reliability

Assay reproducibility is shown in Figure 3. DNA was purified from 96 samples of mouse tail end clippings. To obtain identical sample material for this experiment, individual samples were lysed, pooled and split to get a homogeneous master lysate. The DNA yield was consistently high around 6.5 µg with a CV of 11 %. The data highlights the robustness and consistency of the automated procedure.

Figure 4 compares typical yields obtained from 3 mm of mouse tail end clippings purified by the manual or automated method, the yields are comparable between the two methods.

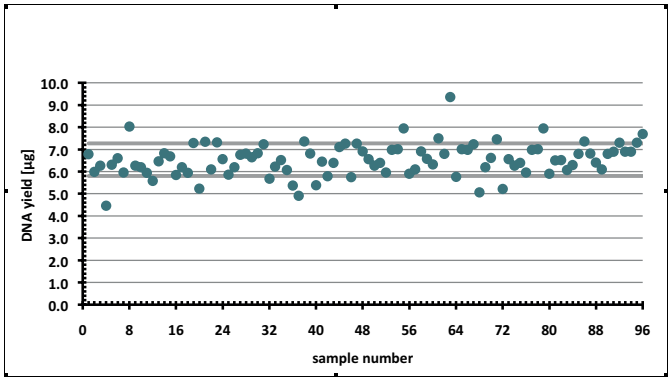

Figure 3 Reproducibility of purification process
DNA was purified from 96 tissue samples from mouse tail end clippings (master lysate). An average DNA yield of 6,5 µg from 3 mm mouse tail clippings (lysed for 6h at 56°C) with a CV of 11 % was obtained.

Downstream applications

The purified DNA is suitable for a broad range of downstream applications, including PCR.

A PCR-based method was chosen to demonstrate the quality of the purified gDNA, which was purified by the manual or automated process. An aliquot of the purified gDNA was amplified by PCR targeting a house keeping gene. As shown in Figure 5, the desired PCR product amplified in all samples.

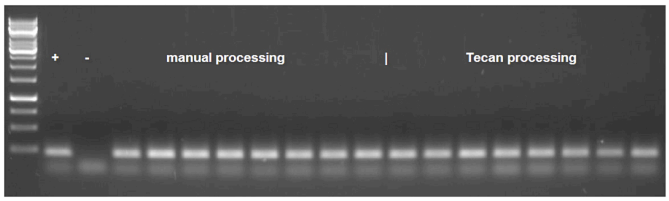

Figure 5 PCR amplification of purified gDNA from mouse tail clippings
Aliquots of the purified gDNA were amplified by PCR targeting a cytoplasmic aconitase gene fragment. Amplified samples were loaded onto a 1 % TAE-agarose gel. The expected 212 bp fragment was obtained in all samples, M: 1 kb size marker (Fermentas) +: pos control, -: negative control.

To demonstrate the sensitivity of the purification process, different quantities of HeLa cells were purified ranging from 10^6 to 10^2 cells. DNA purification was performed using the automated NucleoSpin® Tissue kit protocol, and aliquots (2 µl) of the eluates were subjected to real-time qPCR. The results are illustrated in Figure 6, which shows concentration dependent amplification of the PCR product (colored curves). No amplification could be detected in a negative control sample (grey). High sensitivity was obtained.

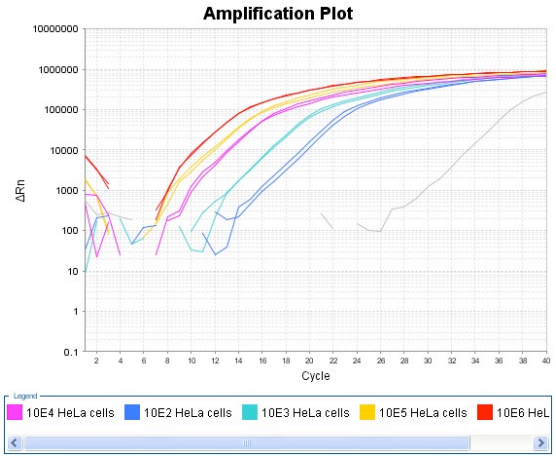

Figure 6 Real-time PCR amplification of DNA purified from HeLa cells 2 µL of gDNA eluate were amplified by PCR (ABI, 7500 Real-Time PCR System, amplification of a 206 bp ATPase6 fragment, SYBR® Green detection , 40 cycles). Specific PCR products were amplified depending on the quantity of starting material (colored curves); no specific PCR product was obtained from the negative control (grey line).

Conclusion

Automation of the NucleoSpin® 96 Tissue kit on a Tecan Freedom EVO® sample preparation workstation enables fast, reliable purification of genomic DNA from tissue in a true walkaway manner, consistently generating high quality DNA. For highest flexibility, or to meet changing laboratory needs, the Tecan 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

Updated list at www.tecan.com\machereynagel

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