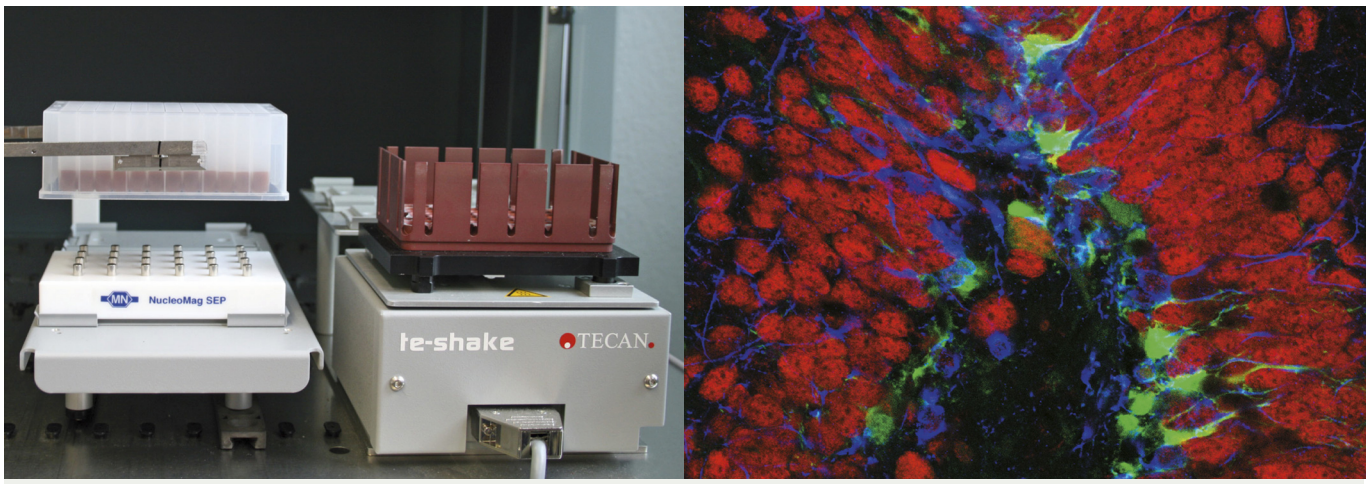

Reliable extraction of highly pure gDNA from tissue

Tissue gDNA extraction with MACHEREY-NAGEL's NucleoMag® 96 Tissue kit and the Freedom EVO® workstation

Introduction

Analysis of tissue and cell samples with molecular diagnostic techniques requires the extraction of highly pure gDNA to assist with the performance of major downstream applications, such as PCR-based genotyping and screening in cancer research. Reliability and reproducibility, as well as the avoidance of any cross-contamination, are of key importance in this field.

Tecan and MACHEREY-NAGEL have joined forces to provide a flexible automated solution for the purification of gDNA from cell and tissue samples. The MACHEREY-NAGEL NucleoMag 96 Tissue kit offers fast purification of highly pure genomic DNA, and is suitable for a broad range of downstream applications, including PCR, real-time PCR and next generation sequencing for research purposes. The purification method is based on magnetic bead separation and can be fully automated on the Freedom EVO platform.

Automation of this application reduces common risks – such as cross-contamination between samples and carry-over of chemicals and solvents – while reducing manual errors and maximizing reproducibility. Full sample tracking further improves overall process security, while increased walkaway times will free staff from repetitive jobs and allow them to perform more highly skilled tasks, increasing the efficiency of their research.

The excellent purity of the extracted DNA is demonstrated by an $A_{260/280}$ ratio greater than or equal to 1.93, as well as excellent real-time PCR performance. Average DNA yields of 7.45 µg are obtained from 10 mg mouse tail end clippings with a low CV of 6.43 %. 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 adaptors and a low level disposable tip ejection option to reduce cross-contamination. Variable throughputs can be achieved using the magnetic separator – from MACHEREY-NAGEL, NucleoMag SEP – positioned on a regular microplate carrier, a Te-Shake™ module for heating and mixing the samples and a Robotic Manipulator (RoMa) Arm (Figure 1).

Sample numbers	Up to 96 samples
Batch time	120 mins for 96 samples
Equipment Tecan	• Freedom EVO 100 platform, 8-channel LiHa Arm configured for disposable tips, 1000 µl syringes, stainless steel deck and safety panel set • Microplate carriers, tube, trough and disposable tip carriers • Wash station with waste disposal • Disposable tips (filtered), 1000 µl • 200 µl and 100 ml troughs • Freedom EVOware® Standard software • RoMa Arm • Te-Shake
Equipment MACHEREY-NAGEL	• NucleoMag 96 Tissue kit • Square-well blocks • NucleoMag SEP Magnetic Separator

Table 1 Overview of equipment for high throughput gDNA purification

Automated workflow

Typically, lysed samples of up to 20 mg of tissue or 1 x 10^7 cells are placed onto the platform and the genomic DNA is purified without any user intervention.

Full automation of the gDNA purification procedure includes lysis of the samples (optional, for example for processing cultured cells), binding of genomic DNA to NucleoMag beads, stringent wash steps and final elution of the purified DNA in volumes of 50 to 200 µl of elution buffer, depending on the subsequent downstream applications.

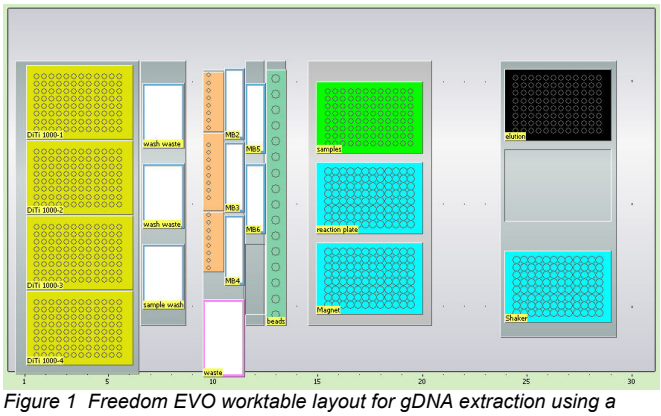

Figure 1 Freedom EVO worktable layout for gDNA extraction using a NucleoMag SEP magnetic separator, Te-Shake module and RoMa Arm.

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

Results

Automation of the NucleoMag 96 Tissue kit on the 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 10^7 cultured cells or bacteria. Fully automated extraction of gDNA from 96 lysates of tissue samples takes only 120 mins.

Yield and reproducibility

DNA yields strongly depend on the type of tissue analysed, and can be as high as 21.1 µg gDNA from 10 mg of liver tissue (see Figure 2).

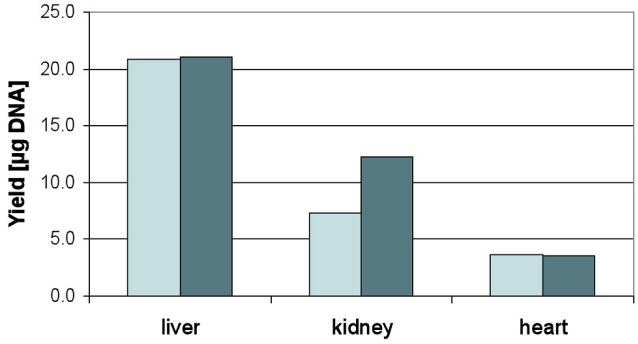

Figure 2 Yield of genomic DNA isolated from different mouse tissues, including liver, kidney and heart. 10 mg of tissue sample was processed. Each bar represents the average of eight samples.

An average yield of 7.45 µg gDNA isolated from mouse tail lysate – representing 10 mg mouse tail per sample – was achieved. The yield was highly reproducible with a low CV of 6.53 % (Figure 3).

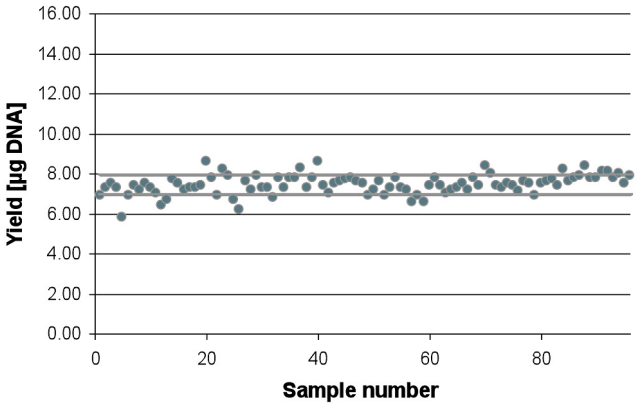

Figure 3 Reproducibility of the purification process. gDNA was purified from mouse tail lysate, representing 10 mg mouse tail end clippings per sample.

Purity

The automated method produces isolated DNA of excellent purity. Genomic DNA was isolated from different mouse tissue lysates. The A260/280 ratio obtained by automated processing on the Freedom EVO workstation is generally higher than for the manual method, with average ratios greater than or equal to 1.93 (Figure 4).

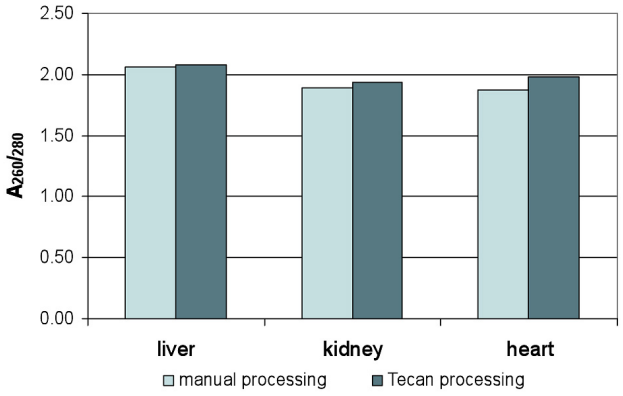

Figure 4 Excellent purity of genomic DNA isolated from different mouse tissues, including liver, kidney and heart. 10 mg of tissue sample was processed. Each bar represents the average of eight samples.

Downstream applications and cross-contamination

The purified DNA is suitable for a broad range of downstream applications, including PCR. A real-time PCR-based method was chosen to demonstrate the high quality of the purified gDNA. A 1:10 dilution of each gDNA eluate was amplified by PCR (ABI, 7500 Real-Time PCR System, cytoplasmic aconitase, 212 bp fragment, 40 cycles). Specific PCR products were amplified from gDNA samples from all tested tissues.

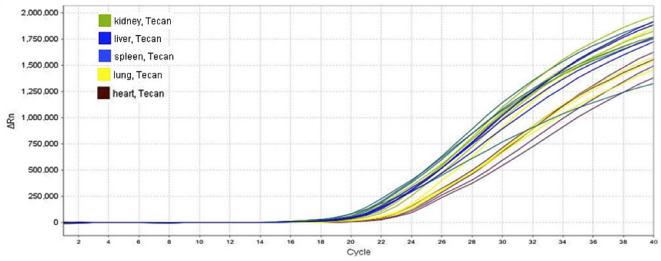

Figure 5 qPCR, automated processing. Eluates were diluted 1:10 prior to qPCR.

To demonstrate the sensitivity of the purification process, tissue samples (prepared from a master lysate and representing 10 mg mouse tail each) and PBS buffer were loaded into a square-well block in a checkerboard pattern and gDNA was purified. The resulting gDNA eluate was diluted 1:100 and amplified by PCR (ABI, 7500 Real-Time PCR System, GADPH, 191 bp fragment, 40 cycles). Specific PCR products were amplified only from the wells containing tissue samples (Figure 6, red curves). No specific PCR product was obtained from the negative control wells filled with PBS buffer (Figure 6, blue curves, unspecific product verified by melting curve analysis), indicating the absence of any cross-contamination.

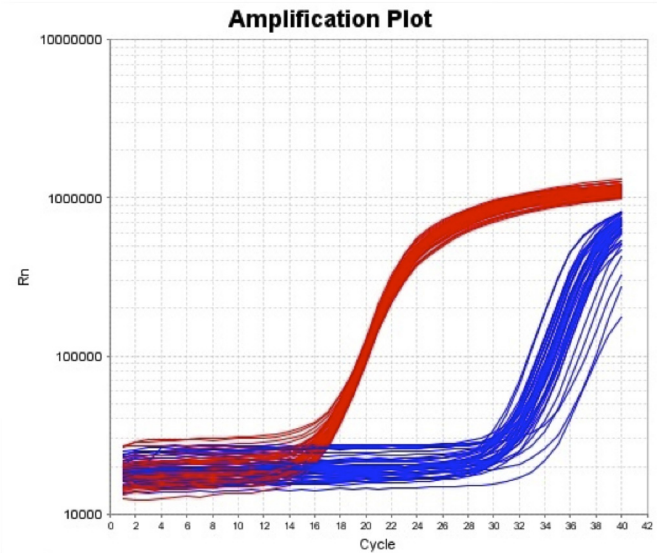

Figure 6 Absence of cross-contamination. Positive gDNA samples (red) and negative control samples (PBS, blue) in a checkerboard pattern were subjected to PCR analysis. gDNA was diluted 1:100 and analyzed in a 40 cycle PCR for the presence of a PCR product (GADPH, 191 bp fragment, ABI, 7500 Real-Time PCR System).

Conclusion

Automation of the NucleoMag 96 Tissue kit on a Tecan Freedom EVO sample preparation workstation enables reliable purification of highly pure genomic DNA from tissue samples. This true walkaway solution will increase the overall productivity of any laboratory.

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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