Fast and convenient high purity gDNA from blood

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

Introduction

Purification of large numbers of blood samples represents a serious bottleneck in sample processing for genotyping or general screening projects. Furthermore reliability, process control and the avoidance of cross-contamination are major issues for the purification of gDNA.

Tecan and MACHEREY-NAGEL have combined forces to provide a flexible automated solution for the purification of gDNA from blood samples for research use only, not for use in clinical diagnostic.
MACHEREY-NAGEL's NucleoSpin® 96 Blood kit for fast extraction of highly pure genomic DNA is suitable for a broad range of downstream applications, such as PCR for HLA typing. 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 from 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 extracted DNA is demonstrated by an average A260/280 ratio of 1.9, in addition to excellent PCR performance. High yields of up to 6 µg gDNA are obtained from 200 µl human blood, stabilized with EDTA, Li-heparin or Na-citrat. 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 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 55 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 Blood kit • Column Holder A (required for 8-well strips only)

Table 1 Overview of equipment for blood extraction

Automated workflow

Whole blood samples of 200 µl are processed directly from sample tubes. They are placed onto the platform and the genomic DNA is purified without any user intervention. The fully automated gDNA purification procedure includes sampling from sample tubes (availability of this step is depending on instrument size), lysis of the samples with proteinase K, binding of genomic DNA to silica membranes, stringent wash steps and the final elution of the 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.

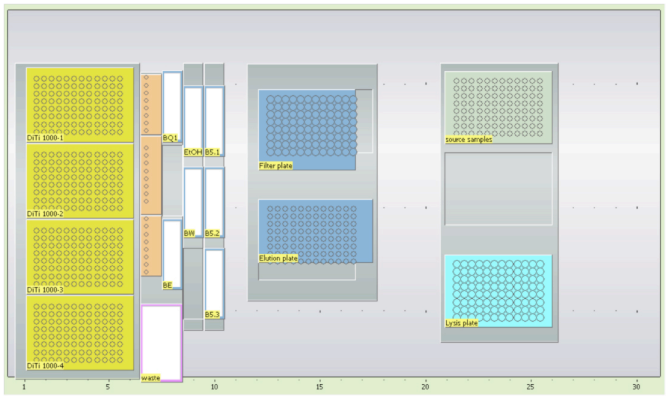

Figure 1 Freedom EVO worktable for blood extraction with the Te-VacS and Te-Shake modules and use of the Robotic Manipulator Arm and the Liquid Handling Arm

Results

Automation of the NucleoSpin® 96 Blood kit on the Tecan Freedom EVO® sample preparation workstation allows fast, convenient and reliable purification of gDNA from a variety of blood sources. Fresh or frozen human whole blood stabilized with EDTA, Li-heparin or Na-citrat can be used.
The automated method produces isolated DNA of excellent purity (Figure 2), and the yield is consistently high (Figure 3). The complete automated purification of 96, 200 µl blood samples takes 1h 55 mins.

DNA Purity

The purity of DNA purified with the MACHEREY-NAGEL NucleoSpin® Blood kit is excellent. With human blood samples A260/280 average ratios of 1.9 were obtained (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 and HLA typing.

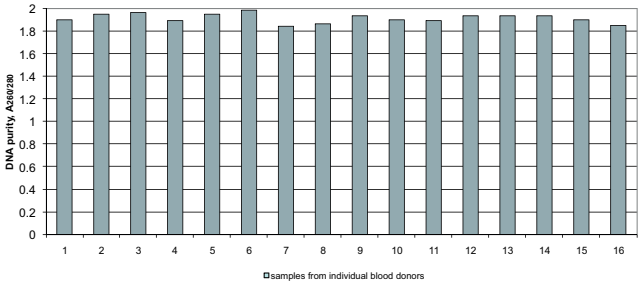

Figure 2 Excellent purity of gDNA isolated from different fresh human EDTA blood samples
DNA was purified from 16 samples (samples 1 –16 in triplicate). Each bar represents the average A260/280 ratio from 200 µl blood samples (n=3). The corresponding DNA yields are shown in Figure 4.

Yield and Reliability

Assay reproducibility and intra-assay variation is shown in Figure 3. A blood pool was used to perform 96 extractions from identical aliquots, giving a DNA yield of 2.5 µg with a CV of 7.5 %. The data highlights the robustness and consistency of the automated procedure.

Figure 4 shows typical yields obtained from individual samples of 200 µl human EDTA blood.

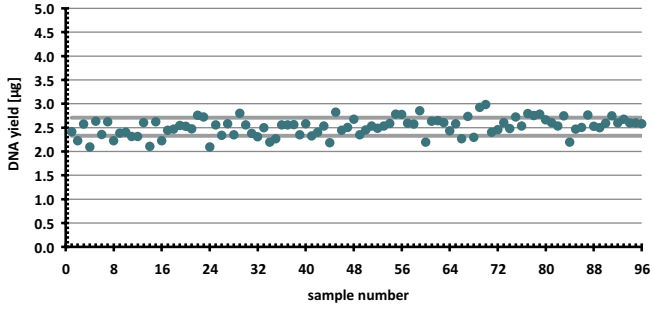

Figure 3 Reproducibility of DNA purifcation process
DNA was purified from pooled frozen EDTA blood samples. An average DNA yield of 2.5 µg was obtained from 200 µl frozen blood. The CV of 7.5 % demonstrates the high reproducibilty of the purifcation process.

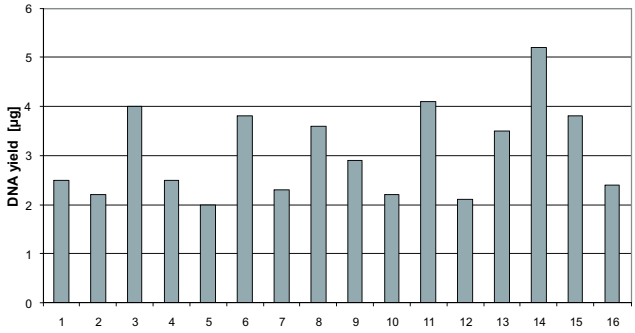

Figure 4 DNA isolation from fresh human blood samples
DNA was isolated from 16 individual fresh human EDTA blood samples (Sample 1 –16). Each bar represents the average from 200 µl blood samples (n=3).

Downstream applications and cross-contamination

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 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 was amplified in all samples.

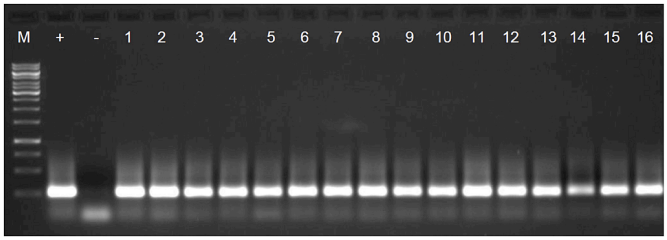

Figure 5 PCR amplification of eluted DNA
Aliquots of the purified gDNA (n = 16) were amplified by PCR targeting a beta-Actin gene fragment. Amplified samples were loaded onto a 1 % TAE-agarose gel. The expected 200 bp fragment was obtained in all samples (1-16), M: 1 kb size marker (Fermentas) +: pos control, -: negative control.

To demonstrate the absence of cross-contamination 48 blood samples, plus PBS buffer as negative controls, were arranged in a square-well block in a checkerboard pattern. DNA isolation of both positive and negative samples was performed using the automated NucleoSpin® Blood kit protocol, and aliquots (2 µl) of the eluates were subjected to real-time qPCR. The results are illustrated in Figure 6, which shows positive (red) and negative (blue) samples. No amplification could be detected in the negative samples, indicating that there was no cross-contamination during this experiment.

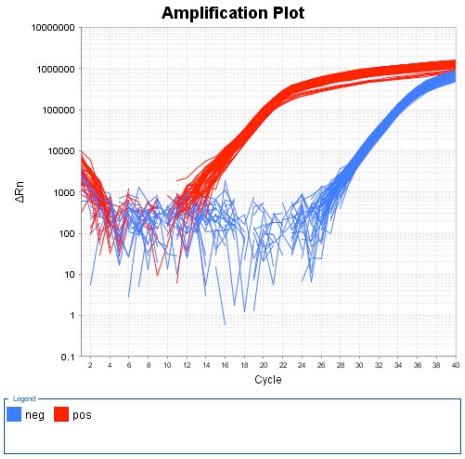

Figure 6 Cross-contamination analysis
2 µl of gDNA eluate were amplified by PCR (ABI, 7500 Real-Time PCR System, beta-Actin, 200 bp fragment, SYBR® Green detection, 40 cycles). Specific PCR products were amplified only from the wells which were filled with blood samples (red curves), no specific PCR product was obtained from the wells filled with PBS buffer (blue lines). No cross contamination was observed.

Conclusion

Automation of the NucleoSpin® 96 Blood kit on a Tecan Freedom EVO® sample preparation workstation enables fast, reliable extraction of genomic DNA from human blood 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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