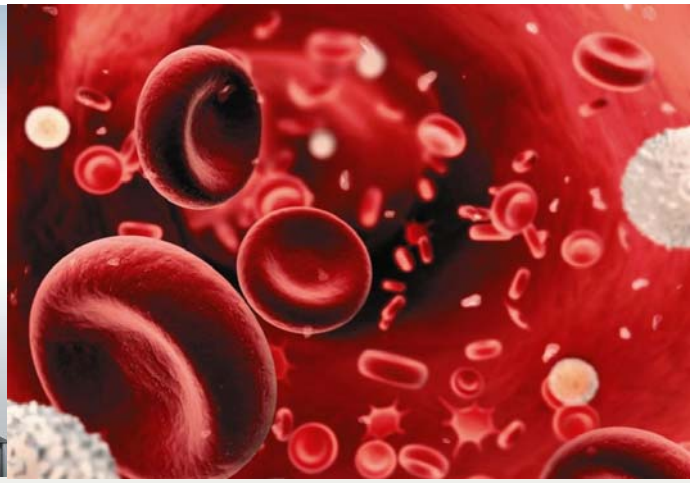

Fast, efficient blood purification

Reliable, high yield extraction of exceptional purity genomic DNA from blood samples, using the NucleoMag® Blood 200 µL 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, and reliability, process control and the avoidance of cross-contamination are major issues for the extraction of gDNA.

Tecan and MACHEREY-NAGEL have combined forces to provide a flexible automated solution for the purification of gDNA from blood samples. MACHEREY-NAGEL's NucleoMag Blood 200 µL kit for fast extraction of highly pure genomic DNA is suitable for a broad range of downstream applications, such as PCR. The extraction method, for research use, is based on the use of magnetic beads 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 tasks, 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 $A_{260/280}$ 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. Animal blood can also be used. Full automation of the gDNA purification process on a Freedom EVO workstation streamlines laboratory workflows and provides reliable, fast extraction of highly pure gDNA.

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 adapters and low level disposable tip ejection options to reduce cross-contamination. A Robotic Manipulator (RoMa) Arm transfers the samples on and off the NucleoMag SEP Magnetic Separator from MACHEREY-NAGEL. 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	3 h 10 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-Shake • Microplate, trough, tube and disposable tip carriers • Wash station with waste disposal • Disposable tips (filtered) 1000 µl, 200 µl and 100 ml troughs • Freedom EVOware® Standard software package
MACHEREY-NAGEL Equipment	• NucleoMag Blood 200 µL kit • Square-well blocks • NucleoMag SEP Magnetic Separator

Table 1 Overview of equipment required for blood extraction.

Automated workflow

Samples (200 µl) of whole blood are placed onto the robot and the genomic DNA is purified without any user intervention. The fully automated gDNA purification procedure includes lysis of the samples, binding of genomic DNA to magnetic beads, 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.

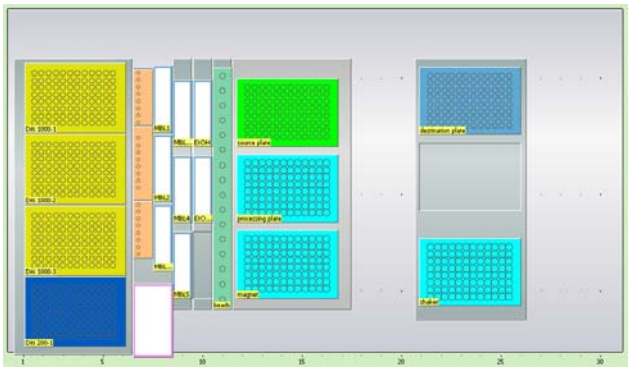

Figure 1 Worktable layout for high throughput isolation of DNA from whole blood using Tecan's Te-Shake and MACHEREY-NAGEL's magnetic separator.

Results

Automation of the NucleoMag Blood 200 µL 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, or animal blood (eg. from horses) can be used. Manual and automated methods produce isolated DNA of comparable excellent purity (Figures 2 and 3), and the yield is consistently high (Figure 4). The complete automated extraction of 96, 200 µl blood samples takes 3h 10 mins.

Purity

The purity of DNA extracted with MACHEREY-NAGEL's NucleoMag Blood 200 µL kit is excellent. With human blood sample $A_{260/280}$ ratios averaging 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. The automated method for the NucleoMag Blood 200 µL kit even performs well with more challenging samples, for example horse blood, giving very good $A_{260/280}$ ratios of around 1.8 (data not shown).

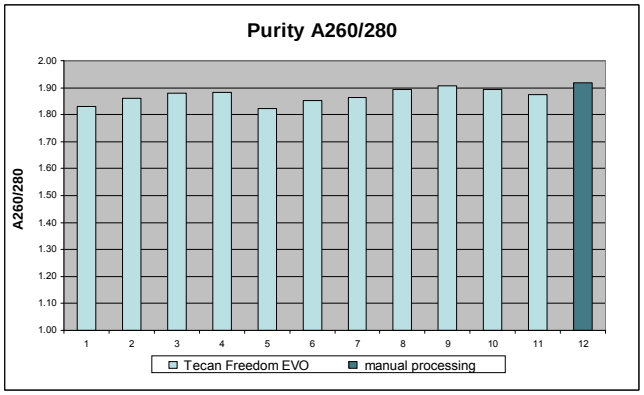

Figure 2 Purity of gDNA isolated from human whole blood with the automated and manual method.. Each bar represents the average $A_{260/280}$ ratio from 200 µl samples of blood (n=8).

Quality

High molecular weight DNA was obtained, demonstrated by the analysis of purified samples using agarose gel electrophoresis. A distinct high molecular weight band was obtained and no degradation of DNA was observed.

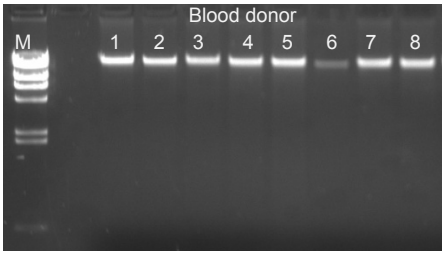

Figure 3 DNA quality; DNA was isolated from eight different blood donors. 10 µl of eluted DNA samples were loaded on a 1 % TAE-agarose gel. A l/Hind III size marker (Fermentas) was used as a standard.

Yield and Reliability

Assay reproducibility and intra-assay variation is shown in Figure 4. A blood pool was used to perform 96 extractions from identical aliquots, giving a DNA yield of 6 µg with a CV of 10 %. The data highlights the robustness and consistency of the automated procedure.

Figure 5 shows typical yields obtained from individual samples of 200 ul human whole EDTA blood. Equal or higher yields were obtained with the automated method for most samples. Yields obtained from more challenging samples, like horse blood, are also high as shown in Figure 6.

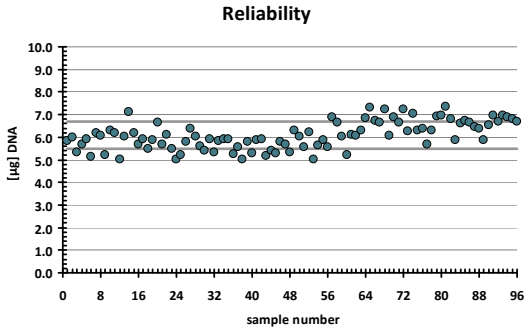

Figure 4 DNA isolation from a pooled blood sample. DNA yield was 6 µg with a CV of 10 %.

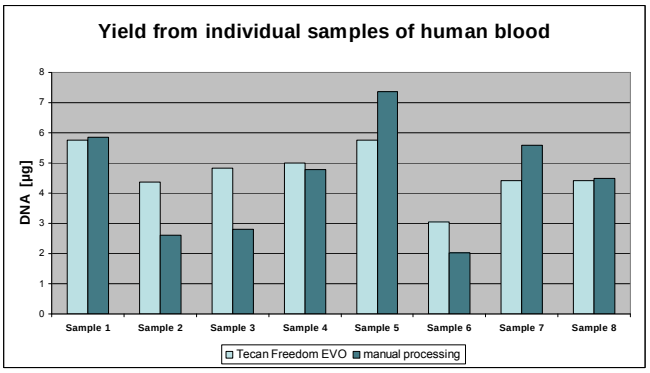

Figure 5 DNA yields from individual samples of 200 ul human whole blood ranges between 2 ug to 7 ug.

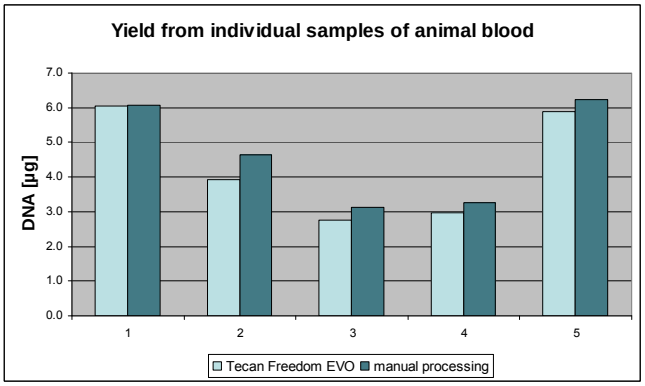

Figure 6 DNA yields from individual samples of 200 ul horse blood ranges between 3 ug to 6 ug.

Downstream applications and cross contamination

The purified DNA is suitable for a broad range of downstream applications, including PCR. A PCR based method was also chosen to analyze potential cross-contamination during the automated extraction process. 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 NucleoMag Blood 200 µl kit protocol, and aliquots (2 µl) of the eluates were subjected to real-time qPCR. The results are illustrated in Figure 7, which shows twelve positive (blue) and twelve negative (black) samples. No amplification could be detected in the negative samples, indicating that there was no cross-contamination during this experiment.

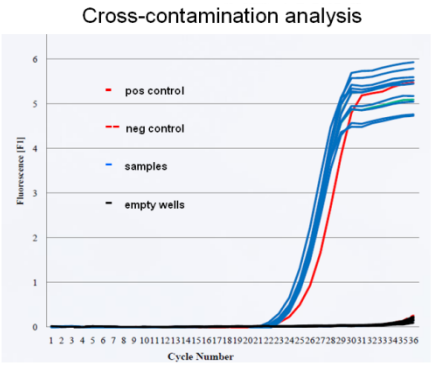

Figure 7. Absence of cross-contamination. Twelve positive and twelve negative DNA samples were selected randomly and subjected to PCR analysis. 2 µl of eluted DNA were analyzed in a 36 cycle PCR for the presence of a PCR product (beta Actin,200 bp fragment, Roche LightCycler®).

The amplified PCR products were loaded onto a 1 % agarose gel (Figure 8). Once again, no amplified product was seen in those wells that did not contain a blood sample (-), whereas the wells that contained a blood sample (+) showed a clearly defined amplified fragment.

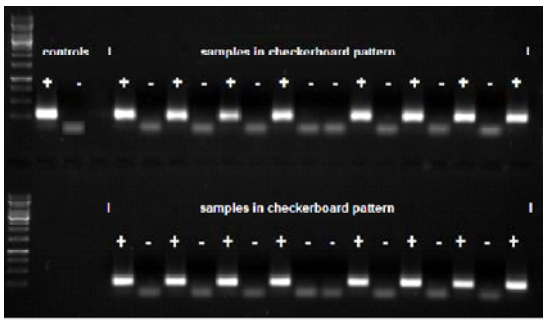

Figure 8. No cross-contamination was observed during gDNA extraction using the automated method. Blood samples were loaded into a square-well block in a checkerboard pattern and gDNA was purified. Subsequently, a fragment of the gDNA was amplified by PCR and loaded on a 1 % agarose gel. A 1 kb size marker (Fermentas) was used as a standard.

Conclusion

Automation of the NucleoMag Blood 200 µL kit on a Tecan Freedom EVO sample preparation workstation enables fast, reliable extraction of genomic DNA from human and animal 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

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

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