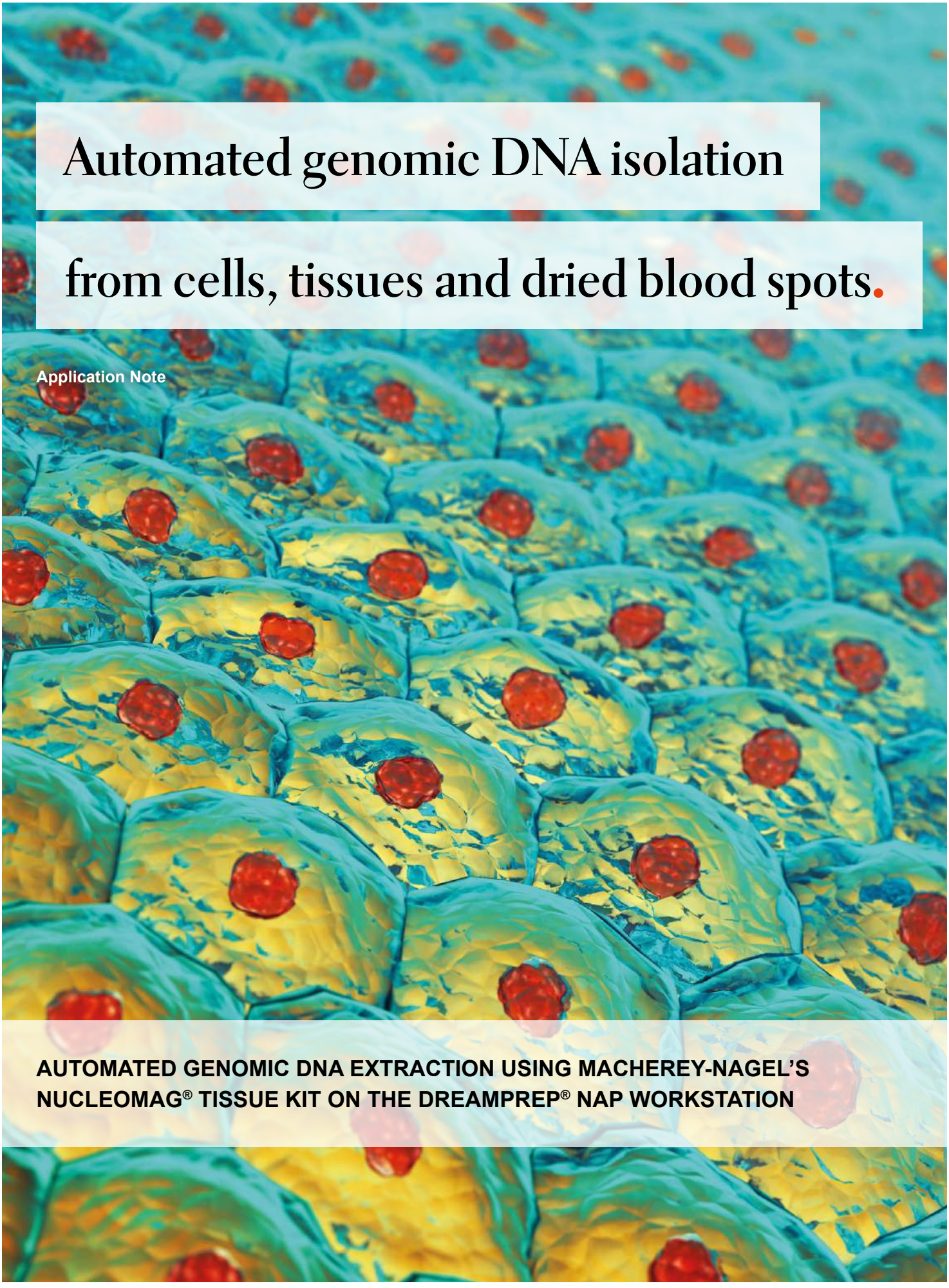
Automated genomic DNA isolation from cells, tissues and dried blood spots.

Application Note



AUTOMATED GENOMIC DNA EXTRACTION USING MACHEREY-NAGEL'S NUCLEOMAG® TISSUE KIT ON THE DREAMPREP® NAP WORKSTATION

TECAN.

INTRODUCTION

Genomic DNA (gDNA) constitutes the total genetic information of an organism. This genetic information is of great interest for answering a wide variety of questions in life sciences research – as well as for applied testing in medicine and biotechnology – using techniques such as genomic mapping, sequencing, and screening of genomic libraries for gene identification. Molecular biology laboratories require pure and high quality DNA that is free of impurities for these demanding downstream analyses, making efficient isolation of gDNA from cells or tissue samples essential.

Tecan has joined forces with MACHEREY-NAGEL to demonstrate a flexible automated solution for the purification of gDNA from a variety of cell and tissue samples. This application note demonstrates a fully automated gDNA purification workflow for MACHEREY-NAGEL's proven NucleoMag Tissue kit on the DreamPrep NAP workstation. Quantification and quality control of the extracted nucleic acids were performed with the integrated Frida Reader™ module. The extracted gDNA was of high purity and suitable for a broad range of common downstream applications, such as end-point PCR, qPCR and library preparation for NGS.

MATERIALS AND METHODS

The automated gDNA extraction workflow was performed using the DreamPrep NAP system, which is based on the Fluent® 480 Automation Workstation. The system was configured for nucleic acid extraction workflows using magnetic bead-based procedures, in combination with the FluentControl™ GX Assurance Software. The DreamPrep NAP was equipped with a Flexible Channel Arm™ for parallel processing of eight samples, a Robotic Gripper Arm™, Fluent ID™ and handheld barcode scanners for sample and reagent identification, 16 tip box positions, a BioShake™ D30-T elm (QInstruments) for heating and shaking, and a NucleoMag SEP (MACHEREY-NAGEL) magnetic separator (Figure 1). The integrated TouchTools™ module allowed for easy method start-up, and provided user-friendly worktable loading instructions. The protocol was then executed without any further user interaction, demonstrating a fully walkaway solution.

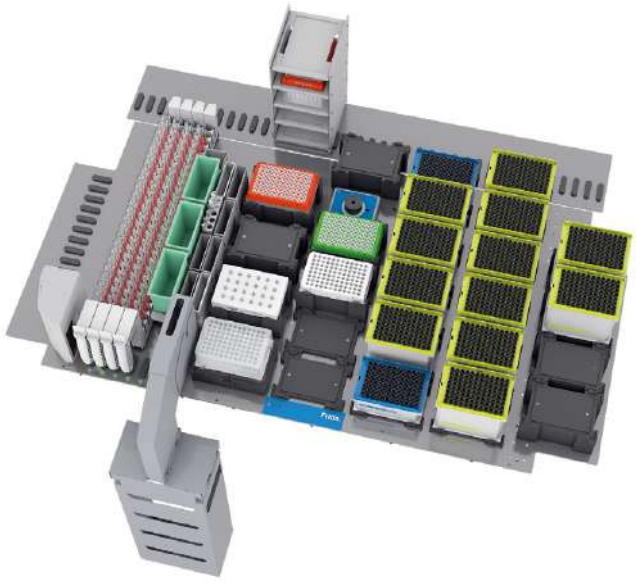


Figure 1: DreamPrep NAP system featuring MACHEREY-NAGEL worktable configuration. The Frida Reader™ module can be integrated directly into the worktable for direct nucleic acid quantification and quality control using UV absorbance-based measurements, without any sample loss.

The tailored protocol enables the processing of up to 96 samples per run. gDNA extraction was carried out using the NucleoMag Tissue kit, which allows rapid and automated nucleic acid purification from a variety of human and animal tissue samples, dried blood spots or cultured cells.

The isolation procedure of the NucleoMag Tissue kit is based on reversible adsorption of nucleic acids to paramagnetic NucleoMag B-beads under appropriate binding conditions. Up to 20 mg tissue, 1 × 10^6 cells, or dried blood spots were mixed and incubated with proteinase K and lysis buffer T1. Following lysis (lysate clarification is optional for leftover solids from tissue lysis), the lysate was loaded onto the DreamPrep NAP automation platform. The reversible binding of nucleic acids in the lysate to paramagnetic beads was achieved by adjustment with binding buffer MB2 (MACHEREY-NAGEL). After the magnetic separation, the NucleoMag B-Beads were washed to remove contaminants and salts using wash buffers MB3 and MB4. After air drying, highly pure nucleic acids were eluted in 50 to 200 µl of elution buffer MB6 (elution volume may vary depending on downstream application) (Table 1).

After elution, the integrated Frida Reader allowed onboard quantification and normalization of the extracted nucleic acids, delivering normalized, pure gDNA and quantification results for each sample. A standard microvolume plate

reader (a Take3 microvolume plate on a BioTek Synergy™ HT reader) was used to run performance comparisons. Downstream qPCR analysis was carried out using a TaqMan® probe for a 250 bp ß-actin amplicon and the SensiFAST™ Probe Lo-ROX Kit (Meridian Bioscience) for a 77 bp GAPDH amplicon on an Applied Biosystems® 7500 Real-Time PCR System (Thermo Fisher Scientific). To check for DNA integrity, agarose gel electrophoresis was performed (~500 ng DNA per gel lane; 1 % TAE-agarose gel; DNA size standard: Lambda Hind III Marker).

NucleoMag Tissue	
Technology	Magnetic beads
Sample material	<20 mg animal tissue <20 mg human tissue 10^6 cultivated cells Dried blood spots
Target molecules	Genomic DNA
Fragment size	300 bp to approx. 50 kbp
Elution volume	50-200 µl

Table 1: NucleoMag Tissue kit product description.

RESULTS AND DATA ANALYSIS

Isolation of gDNA from HeLa cells

gDNA was isolated from different amounts of HeLa cells (1 x 10^3 to 1 x 10^6 cells) using the NucleoMag Tissue kit on the DreamPrep NAP workstation. qPCR analysis was subsequently performed, and the results demonstrate reliable detection of gDNA material (Figure 2).

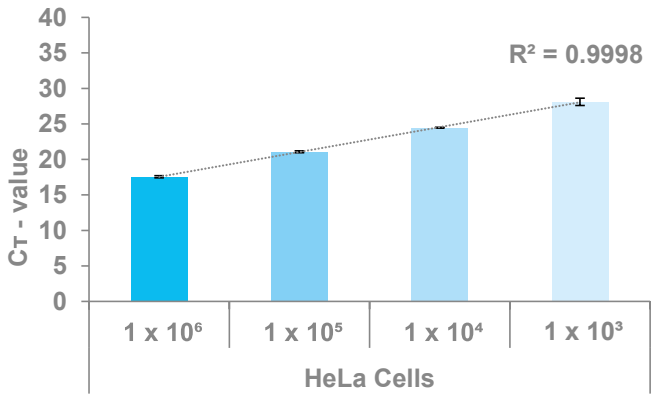


Figure 2: Isolation of gDNA from HeLa cells. gDNA was isolated from different amounts of HeLa cells. The qPCR results demonstrate a reliable detection of gDNA with perfect linearity in the 10-fold dilution raw of sample cells (R^2 = 0.9998).

DNA extraction from dried blood spots

Human blood was sampled using MACHEREY-NAGEL's NucleoCard® blood storage cards. The special chemical impregnation of these filter paper cards stabilizes blood samples and prevents nucleic acid degradation during long-term storage or sample shipment, even at ambient temperature. Different numbers of punches from recently dried blood spots (Figure 3A) were lysed in the presence of proteinase K and lysis buffer T1, then and subsequently purified using the DreamPrep NAP automation platform. qPCR analysis was then performed. The results show a consistently high gDNA yield for all tested blood samples (n=4) after a two-fold dilution. Comparable results were gained even for older samples, demonstrating the reliable preservation and recovery of gDNA even after long-term storage (Figure 3B).

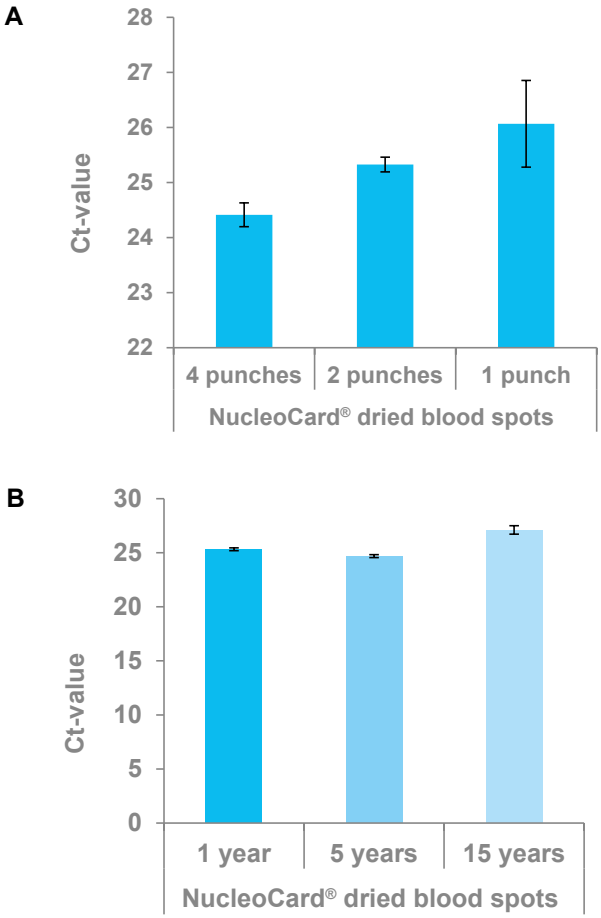


Figure 3: DNA extraction from dried blood spots.

(A) One, two and four punches from NucleoCard dried blood spots were lysed. The qPCR results form a two-fold dilution demonstrated consistently high gDNA yields for all samples tested (n=4).

(B) Detection of the ß-actin amplicon in gDNA after purification from NucleoCard dried blood samples stored for one, five or fifteen years. The comparable results demonstrate the reliable preservation and recovery of gDNA, even after long-term storage.

Isolation of DNA from different animal tissues

DNA was isolated from mouse, pig, sheep and deer tissue samples using the NucleoMag Tissue kit on the DreamPrep NAP workstation. UV spectrometry was used to determine the purity of the extracts. The results showed the high purity for the gDNA eluates, with averages of 1.84 ± 0.01 for A_{260}/A_{280} (blue bars) and 1.95 ± 0.06 for A_{260}/A_{230} (orange squares) (Figure 4A). Concentrations of total gDNA extracted ranged between 3.3 ± 0.14 µg (2.5 mg mouse tail tissue) and 9.83 ± 0.09 µg (5 mg sheep kidney tissue). Additionally, a qPCR analysis (Figure 4B, orange squares) was performed with a TaqMan probe for a 250 bp ß-actin amplicon (pig, sheep, deer) or for a 77 bp GAPDH amplicon (mouse) (Figure 4B). The results showed reliable detection of the target amplicon in all samples, and demonstrating the suitability of the purified nucleic acids for common enzyme-based downstream applications.

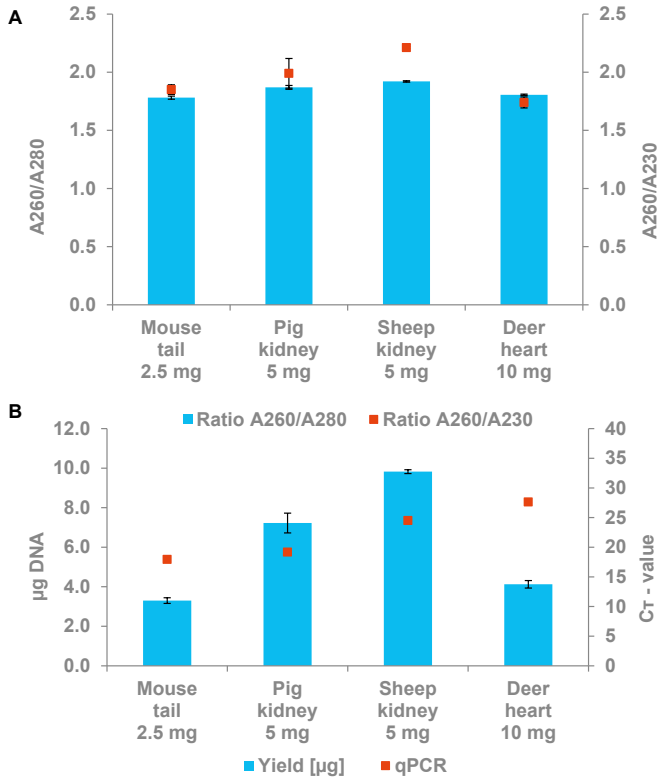


Figure 4: The high purity of isolated DNA from different animal tissues (including mouse, pig, sheep and deer samples) using the NucleoMag kit on the DreamPrep NAP workstation.

(A) The purity of gDNA eluates.

(B) The tissue- and sample-dependent total DNA yields. Subsequent PCR analysis (B, red squares) was performed, demonstrating the suitability of purified nucleic acids for common enzyme-based downstream applications.

Determination of DNA integrity via agarose gel electrophoresis

The integrity of the isolated gDNA was evaluated via agarose gel electrophoresis, which demonstrated that the gDNA possessed high integrity and purity (Figure 5).

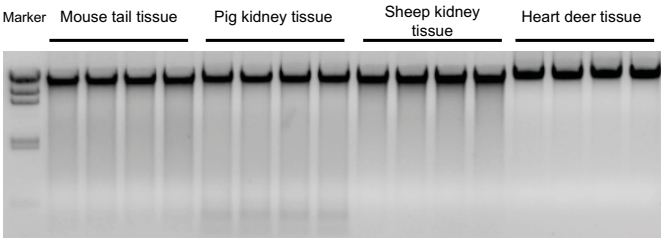


Figure 5: Determination of DNA integrity via agarose gel electrophoresis.

Note: If RNA-free DNA is crucial for downstream applications, an optional RNase digest may be performed before lysate clearance and binding of DNA to NucleoMag B-beads.

Frida Reader reliably quantifies DNA and RNA samples without sample loss

DNA purity and concentration measurements were highly comparable between the Frida Reader and a standard microvolume plate reader (Figure 6). The Frida Reader offers UV-absorbance based concentration and purity measurements of nucleic acids in a hanging drop, avoiding any loss of sample. The comparative data shows that the Frida Reader measures the same values for purity and total yields when compared to standard methods, without having to consume valuable DNA and RNA sample eluates.

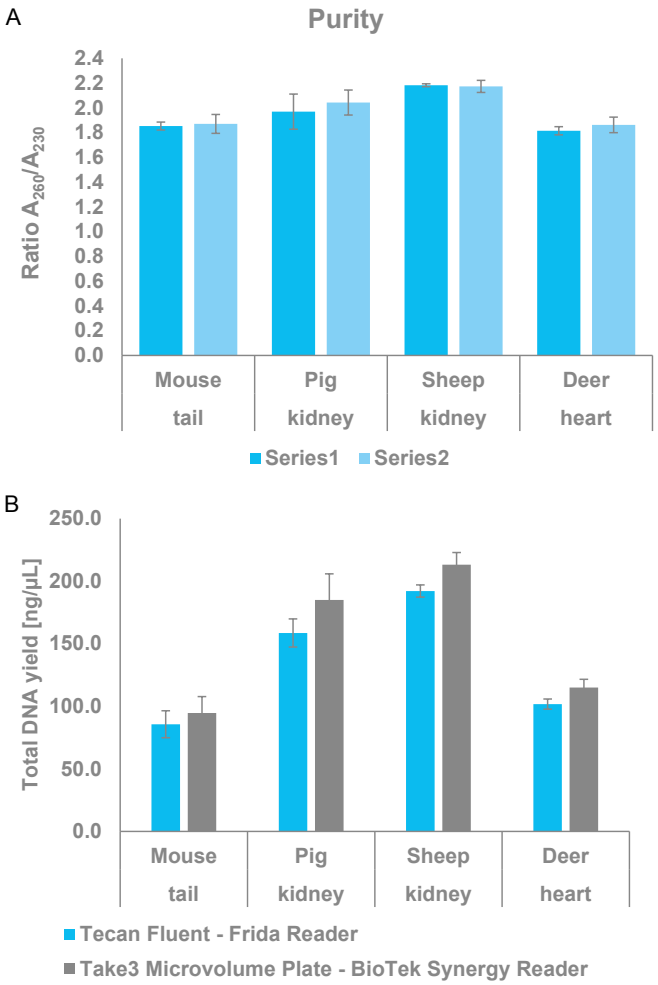


Figure 6: The Frida Reader reliably quantifies DNA and RNA samples without sample loss. DNA purity **(A)** and concentration **(B)** measurements were highly comparable between the integrated Frida Reader and a standard microvolume plate reader.

SUMMARY

MACHEREY-NAGEL and Tecan have collaborated to demonstrate a tailored workflow that meets the demands of high throughput life sciences and applied testing laboratories. The combination of the NucleoMag Tissue kit with the DreamPrep NAP workstation offers streamlined genomic workflows with a verified methodology. This novel workflow is easy and straightforward to perform, with direct quality assessment via the Frida Reader module, saving hands-on time for increased daily throughput. The system is highly flexible, enabling gDNA extraction from various samples – like cultured cells, tissue samples or dried blood spots – with reproducible yields and purities. The resulting purified nucleic acids are suitable for all common downstream applications, such as genotyping, sequencing, parental or husbandry testing, or clinical research.

ACKNOWLEDGEMENTS

This protocol was developed by MACHEREY-NAGEL application scientists, and is intended for research use only. Users are responsible for determining the suitability of the protocol for their application.

LEARN MORE

To learn more about Tecan® nucleic acid purification solutions, contact your sales representative or visit **www.tecan.com/NAP**.

To learn more about MACHEREY-NAGEL's NucleoMag Tissue kit or other solutions for DNA/RNA extraction from diverse sample materials, please visit **www.mn-net.com/bioanalysis**.

About the authors

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