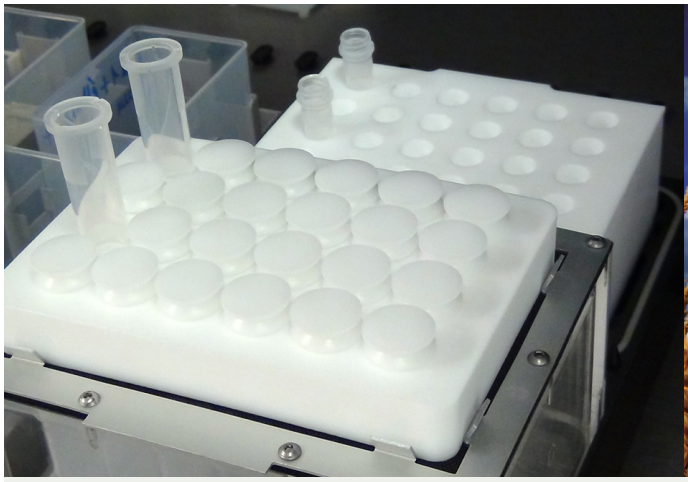

High yields of DNA from plant samples

Reliable extraction of plant DNA using the NucleoSpin® Plant II Midi kit from MACHEREY-NAGEL on a Freedom EVO® platform

Introduction

Sequencing of plant DNA is essential in many crop improvement programs focusing on yield, resistance to pathogens and robustness to other stress factors, such as heat or draught. Sequencing an entire genome, creates the need for purification of high quantities of gDNA. Large volume DNA extraction from plant material is therefore an integral step in plant research.

MACHEREY-NAGEL has developed the NucleoSpin Plant II Midi kit to meet the demand for efficient purification of high quality DNA from a variety of plants and fungi. This solid phase-based extraction process delivers high quality DNA and keeps the workflow very flexible with regard to sample numbers.

Tecan and MACHEREY-NAGEL have joined forces to provide a flexible automated solution for the isolation of plant genomic DNA without compromising yield or purity.

After initial homogenization of the plant material, the workflow can be completely automated on a Freedom EVO sample preparation workstation, reducing risks such as contamination, carry-over and manual errors to a minimum. Sample tracking further increases both sample and overall process security.

Processing time is about 1.5 h for 24 samples from up to 400 mg of plant starting material. The $A_{260/280}$ ratio as a typical indicator of nucleic acid purity is generally in the range of 1.9 and typical yields are approximately 80 µg per 200 mg of starting material from fresh wheat leaves. Hence, full automation of the nucleic acid extraction procedure on a Tecan Freedom EVO workstation streamlines laboratories' workflows and allows for reliable and high yields without compromising quality of plant genomic DNA.

Materials and Methods

Equipment

Freedom EVO 100 liquid handling workstation has been optimized for large volume extraction with a Liquid Handling (LiHa) Arm configured for disposable tips, a Robotic Manipulator (RoMa) Arm, a Te-Shake™, and a Te-VacS™ vacuum station (Figure 1).

	Medium throughput
Sample numbers	Up to 24 samples, or multiples of 24
Batch time	About 1 h 30 mins for 24 samples
Equipment Tecan	• Freedom EVO 100 platform, LiHa Arm configured for disposable tips, RoMa Arm, Te-VacS (special adapter, please inquire), Te-Shake, stainless steel deck and safety panel set • Microplate, tube, trough and disposable tip carriers • Disposable tips (1 ml and 5 ml, filtered) and troughs (100 ml) • Freedom EVOware® Standard software package
Equipment MACHEREY-NAGEL	• NucleoSpin Plant II Midi kit

Table 1 Overview of equipment for medium throughput gDNA extraction from plant material.

Automated workflow

It is recommended that the plant material is homogenized using commercially available homogenization devices (eg. Geno/Grinder® from SPEX SamplePrep) to ensure efficient lysis. Following a heated incubation and centrifugation step, the lysates are placed on the Freedom EVO workstation in 24-well format. The fully automated DNA extraction procedure includes binding of the DNA to silica membranes, wash steps, and the final elution of the purified DNA in 200 µl volumes of elution buffer.

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

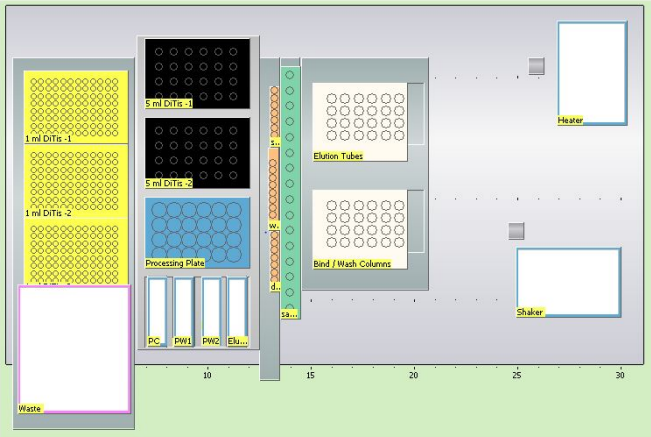

Figure 1 Freedom EVO worktable for gDNA extraction from plant material with a Te-VacS vacuum station, Te-Shake, RoMa and LiHa Arms.

Results

Automation of the NucleoSpin Plant II Midi kit on the Tecan Freedom EVO sample preparation workstation allows convenient and reliable purification of plant genomic DNA. The automated extraction of genomic DNA from up to 400 mg of plant material, such as maize seeds, takes about 1 h 30 mins for 24 samples. Automated DNA extraction on the Freedom EVO workstation and the manual method are comparable with regards to yield and purity.

Purity

The purity of DNA isolated with the MACHEREY-NAGEL NucleoSpin Plant II Midi kit is excellent. An average $A_{260/280}$ ratio of 1.9 was obtained with fresh wheat leaf samples (Figure 2). The eluted DNA is highly pure and free of contaminants, allowing a broad range of downstream applications.

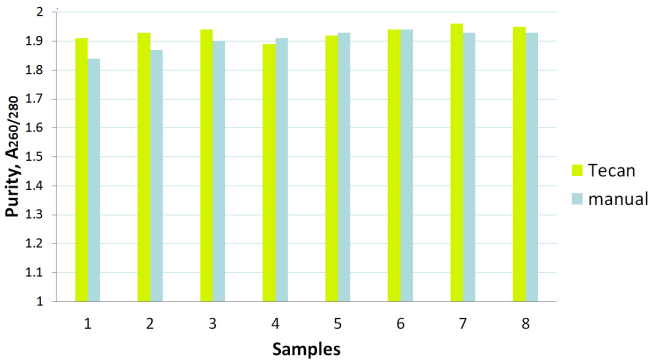

Figure 2 Excellent purity of gDNA isolated from fresh wheat leaves. DNA was purified from eight samples by both the manual and the automated process. Each bar represents the $A_{260/280}$ ratio from a 200 mg sample of fresh wheat leaves.

Yield and reliability

High yields of gDNA are obtained from fresh wheat leaf samples, with an average of 80 µg per 200 mg starting material (Figure 3).

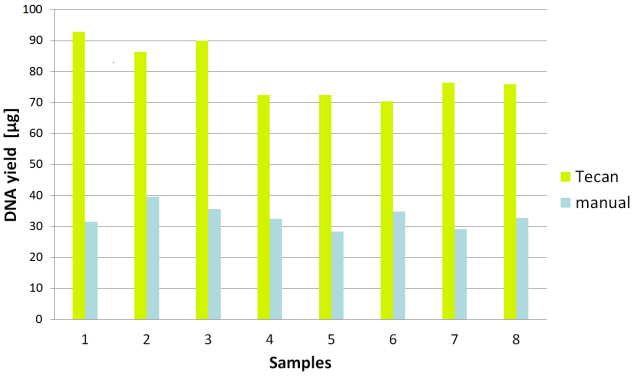

Figure 3 Comparison of the DNA yield from manual and automated processes. DNA was purified from eight samples of 200 mg fresh wheat leaves. An average DNA yield of 80 µg was obtained with the automated process compared to an average yield of 33 µg with the manual process, demonstrating the high efficiency of the automated method.

Assay reproducibility is shown in Figure 4. A pool of plant lysates from different plant species and tissue types was used to perform eight extractions from identical aliquots, and gave consistent gDNA yields with CVs of 3.7, 4.1 and 2.8 %. This data highlights the robustness and consistency of the automated procedure.

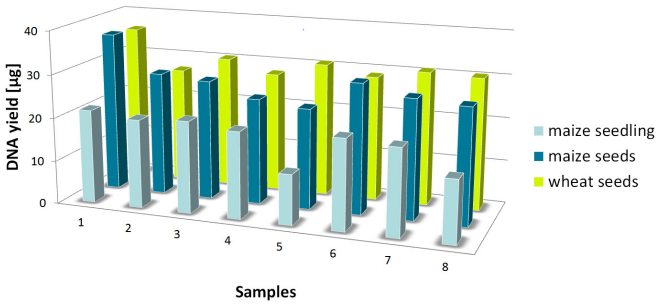

Figure 4 Reproducibility of the DNA purification process. DNA was purified from eight samples of 200 mg maize seedlings, maize seeds and wheat seeds. Average DNA yields of 19 to 30 µg were obtained. The CVs of 3.7, 4.1 and 2.8 % demonstrate the high reproducibility of the purification process.

Downstream applications

A PCR-based method was chosen to demonstrate the quality of the purified gDNA isolated by the automated process. Aliquots of the purified gDNA were amplified by PCR targeting a housekeeping gene, tRNA for leucine. As shown in Figure 5, the desired PCR product was amplified in all samples. The purified DNA is therefore suitable for a broad range of downstream applications, including PCR and real-time PCR.

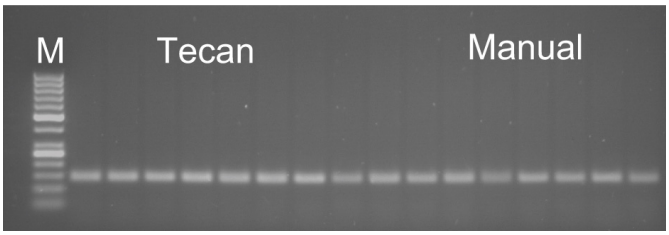

Figure 5 Pure DNA suitable for downstream reaction. 2 µl of gDNA eluate were amplified by PCR specific for a tRNA leucine fragment of around 150 bp. In all samples, a specific product was amplified by both the manual and automated process. M: Lambda DNA/HindIII Marker, 2 (Thermo Scientific).

Conclusion

Automation of the NucleoSpin Plant II Midi kit on a Tecan Freedom EVO sample preparation workstation enables reliable extraction of genomic DNA from plant material in a true walkaway manner. High quality DNA was consistently generated. 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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MACHEREY-NAGEL GmbH & Co. KG
Neumann-Neander-Strasse 6, 52355 Düren, Germany

Further Application Notes

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

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