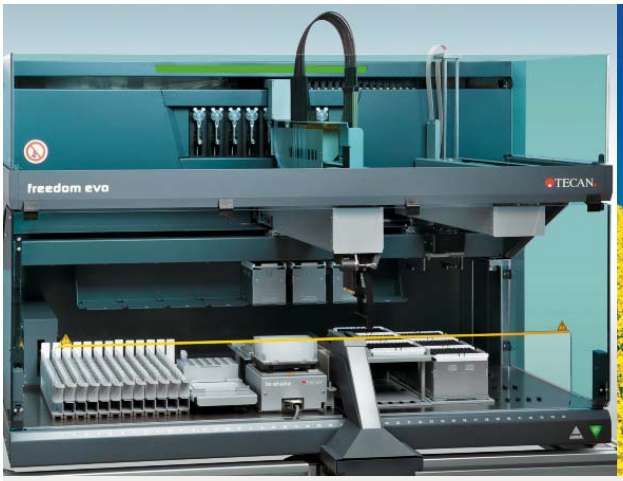

Plant genomics accelerated

Fast and reliable DNA extraction from plants with NucleoMag® 96 Plant from MACHEREY-NAGEL on a Freedom EVO® platform.

Introduction

DNA extraction from plant material is an integral step both in plant research and in food analysis. Plant research is often geared towards crop improvement, focusing on yield, resistance to pathogens and other stress factors, such as heat or draught. Common applications include TILLING (Targeting Induced Local Lesions IN Genomes) and the creation of genetically modified species, as well as traditional breeding technologies. In all cases, breeding success needs to be confirmed not only by phenotyping, but by genotyping as well, creating a need for high throughput genomic DNA extraction.

Similar needs apply to the analysis of plant material in food diagnostics where, for example, the presence or absence of genetic modifications need to be verified.

MACHEREY-NAGEL has developed the NucleoMag 96 Plant kit in order to fullfil the need for fast and homogeneous extraction of high quality DNA from a variety of plants and fungi. The magnetic bead-based extraction process delivers high quality DNA and keeps the workflow very flexible with regard to scalability (amount of starting material) and sample numbers. Tecan and MACHERY-NAGEL have now combined efforts to provide a flexible automated solution for the isolation of genomic plant DNA, without compromising yield or purity.

After initial homogenization of the respective plant material, the workflow can be completely automated on a Freedom EVO workstation, reducing risks such as contamination, carry-over and manual errors to a minimum. Sample tracking improves sample security even more and consequently benefits the overall process security.

Processing time is only 1 hour 30 minutes for up to 48 samples. The 260/280 nm ratio as a standard indicator of nucleic acid purity is generally in the range of 1.72 and typically yields are approximately 8 µg per 50 mg of wheat leaf material, delivering genomic DNA of equal or higher quality compared to manual processing.

Full automation of the nucleic acid extraction procedure on a Freedom EVO workstation streamlines laboratory workflows and provides reliable and fast extraction of high purity plant genomic DNA.

Materials and methods

Equipment

The Freedom EVO liquid handling workstation can be equipped with a 2-, 4- or 8-channel liquid handling arm with disposable tip adaptors and a lower DiTi eject option to reduce cross-contamination. For medium throughputs of up to 48 samples per batch, a Te-MagS™ module is implemented as shown in Figure 1. Higher throughputs can be achieved using a magnetic separator, eg. MACHEREY-NAGEL's NucleoMag SEP positioned on a regular microplate carrier, a Te-Shake™ module for heating and mixing of the samples and a robotic manipulator arm (Fig. 2).

	Medium throughput	High throughput
Sample numbers	1 - 48 samples per batch	1 - 96 samples, or multiples of 96 per batch
Batch time	1 h 30 min	2 h 30 min
Equipment Tecan	• Freedom EVO 100 platform, 8-channel liquid handling arm configured for disposable tips, 1,000 µl syringes, stainless steel deck and safety panel set • tube, trough and disposable tip carriers • washstation with waste • disposable tips (filtered) 1,000 µl, 200 µl and 100 ml troughs • Freedom EVOware® Standard software package	
	• Te-MagS	• robotic manipulator arm • Te-Shake • microplate carriers
Equipment MACHEREY-NAGEL	• NucleoMag 96 Plant kit	• NucleoMag 96 Plant kit • square-well blocks • NucleoMag SEPMagnetic separator

Table 1 Overview of equipment for different throughput requirements

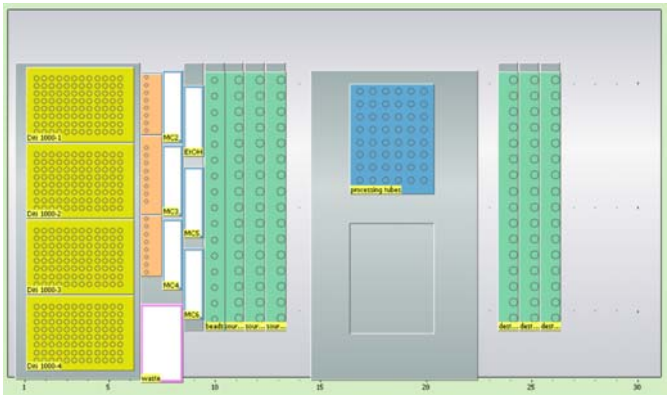

Figure 1 Freedom EVO worktable using the Te-MagS module

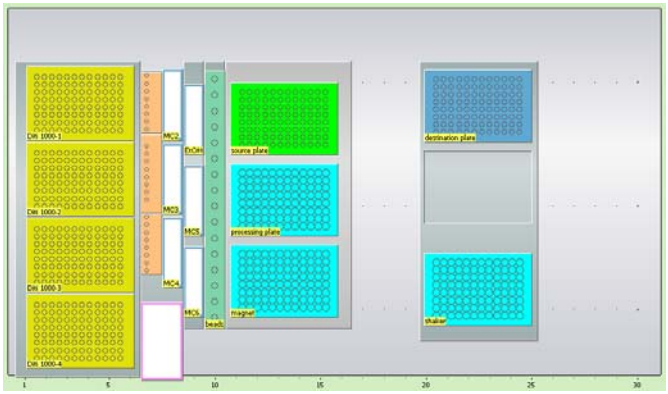

Figure 2 Freedom EVO worktable using the NucleoMag SEP Te-Shake and robotic manipulator arm

Automated workflow

In order to ensure efficient lysis, it is recommended to homogenize the plant material using commercially available homogenization devices (eg. Geno/Grinder® from SPEX SamplePrep). Following an incubation and centrifugation step, the lysates are then introduced into the Freedom EVO workstation either in 96-well format or in 1.5 ml tubes without lids.

The fully automated DNA extraction procedure includes binding of the DNA to the NucloMag Plant beads, followed by wash steps and final elution of the purified DNA in volumes of 50 to 200 µl elution buffer, depending on the further application of the DNA.
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.

Results

Automation of the NucleoMag 96 Plant kit on the Freedom EVO workstation provides fast, convenient and reliable purification of genomic plant DNA.

On average, the manual extraction of genomic DNA from plant material, eg. wheat leaves, takes about 50 minutes for eight samples, whereas the automated process for 48 samples using the Te-MagS takes only one and a half hours.

Automated DNA extraction on the Freedom EVO workstation and the manual method are highly comparable with regards to yield and purity.

Yield

To illustrate homogeneity of the purification process, individual sample lysates were pooled, mixed and split again to obtain a master lysate of identical sample material for automated and manual processing. Each bar of Figures 3 and 5 represents the mean value of eight (one column of the Te-MagS rack) DNA yield and purity values respectively.

As shown in Figure 3, the typical yield of 8 µg per 50 mg of wheat leaf material is equivalent or higher for the automated process compared to the manual process.
The yield from the automated process is very reproducible and consistent (see Figure 4), delivering a perfect result every time.

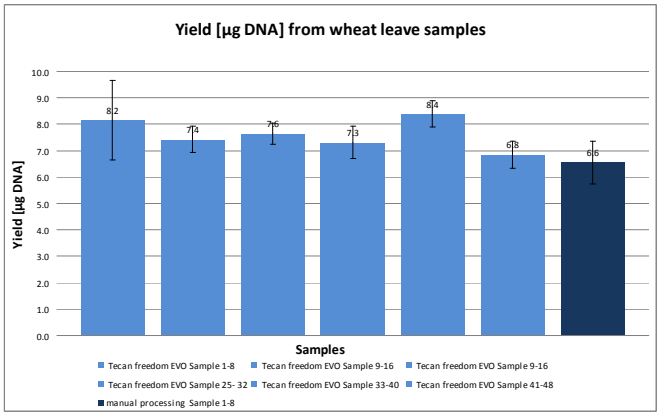

Figure 3 Comparison of the DNA yield using the automated workflow on the Freedom EVO and the manual process.

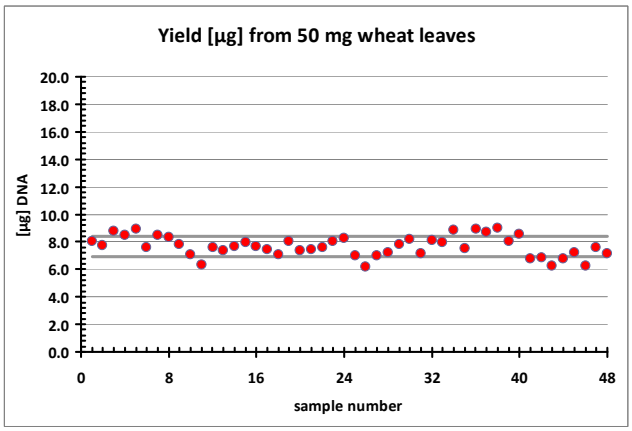

Figure 4 The automated workflow on the Freedom EVO delivers reproducible DNA yields.

Purity

With 260/280 nm ratios of 1.72 on average, the eluted DNA is of excellent purity and is therefore suitable for a full range of downstream applications.

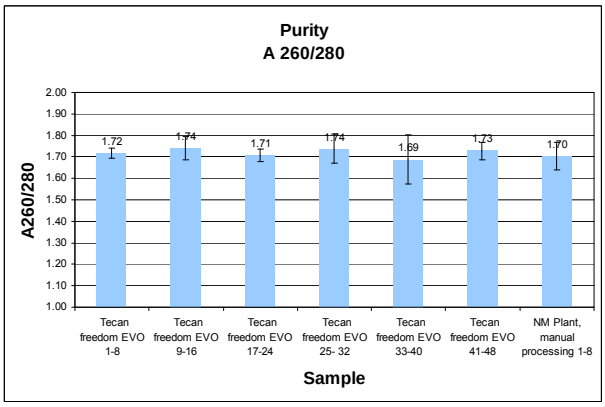

Figure 5 The purity of DNA preparations of automated and manual procedures are very comparable.

The high purity of the extracted DNA is also demonstrated by gel electrophoresis and subsequent staining. DNA from 30 mg of wheat leaf material was purified and eluted in 200 µl elution buffer. 20 µl of the eluate was then subjected to gel electrophoresis as shown in Figure 6.

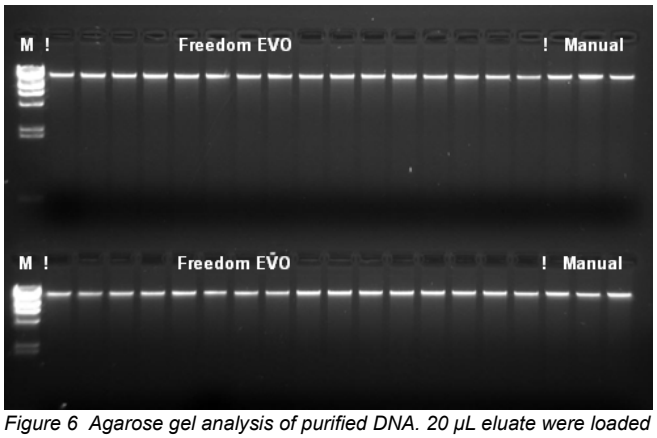

Figure 6 Agarose gel analysis of purified DNA. 20 µL eluate were loaded on a 1 % TAE Gel, M= Marker LamdaHindIIII (Fermentas). DNA of a high molecular weight and high integrity was obtained

The purified DNA is also suitable for a full range of downstream applications, including PCR. This is demonstrated by Figure 7, showing images of PCR based markers used in marker assisted backcrossing (BC) strategy in wheat. DNA extracts of the F1 and F2 population were harvested and analysed with marker barc75, which is linked to the fusarium head blight locus Fhb1, or marker uhw89, which is linked to the Gpc-B1 locus indicating higher grain protein content.

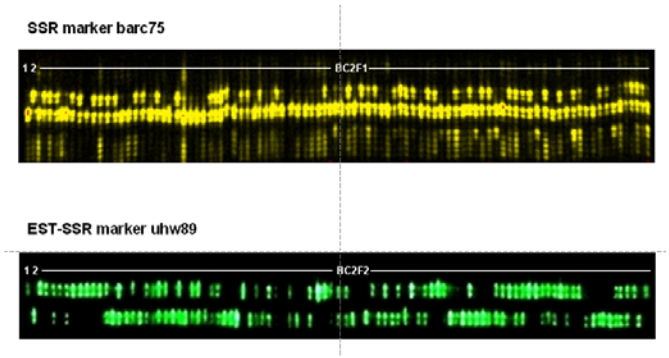

Figure 7 All PCR products were detected on ABI377-96 sequencer (Applied Biosystems). DNA was extracted using MACHEREY-NAGEL NucleoMag 96 Plant on Freedom EVO 150 (4 channel).

Conclusion

Automation of the NucleoMag 96 Plant kit on Tecan's Freedom EVO workstation provides fast and reliable extraction of plant genomic DNA in a true walkaway mode. The combination of the kit and the workstation generates high quality DNA in a consistent manner.

For highest flexibility and changing laboratory needs, the Freedom EVO workstation can be equipped with a number of extension modules, including an absorbance reader, storage modules and cooling devices.

Talk to your local Tecan consultant to adjust the Freedom EVO workstation to your laboratory's needs.

Acknowledgements

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Further application notes

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