Plant genomics at lightning speed

Fast and reliable extraction of plant DNA in 384 format using the NucleoMag® 96 Plant kit on a Freedom EVO® platform equipped with a MultiChannel Arm™ 384

Introduction

Plant research is often geared towards crop improvement, and therefore focuses on yield and robustness 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, the breeding success must be confirmed not only by phenotyping, but also by genotyping, creating a need for high throughput genomic DNA extractions. The analysis of plant material in food diagnostics has similar requirements, for example where the presence or absence of genetic modifications needs to be verified. DNA extraction from plant material is therefore an integral step in both plant research and food analysis.

MACHEREY-NAGEL has developed the NucleoMag 96 Plant kit to meet the demand for fast and homogeneous extraction of high quality DNA from a variety of plants and fungi. This magnetic bead-based extraction process delivers high quality DNA in 384 format and keeps the workflow very flexible with regard to scalability (the amount of starting material) and sample numbers.

Tecan and MACHEREY-NAGEL have joined forces to provide a flexible automated solution for the isolation of genomic plant DNA without compromising yield or purity. After the initial homogenization of the plant material, the workflow can be completely automated on a Freedom EVO sample preparation workstation, reducing the risk of contamination, carry-over and manual errors to a minimum. Sample tracking further increases both sample and overall process security.

Processing time is only 50 min for up to 384 samples of plant extracts. 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 0.1 µg per 7.5 mg of starting material from fresh maize leaves. Hence, full automation of the nucleic acid extraction procedure on a Tecan Freedom EVO workstation streamlines laboratories' workflows and allows for reliable and fast extraction of high quality plant genomic DNA.

Materials and Methods

Equipment

Highest throughputs are achieved using a Freedom EVO 200 liquid handling workstation with a MultiChannel Arm 384 (MCA 384) configured for disposable tips, a Robotic Manipulator (RoMa) Arm and a Te-Stack™ module for disposable tip racks (Figure 1). The workstation also requires a magnetic separator positioned on a regular microplate carrier, and a microplate shaker for sample mixing.

	Ultra-high throughput
Sample numbers	Up to 384 samples, or multiples of 384
Batch time	50 mins for 384 samples
Equipment Tecan	• Freedom Freedom EVO 200 platform, MCA 384 configured for disposable tips, RoMa Arm, Te-Stack configured for disposable tips, stainless steel deck and safety panel set • Microplate, trough, disposable tip and MCA 384 head adapter carriers • Disposable tips (50 µl and 125 µl, filtered) and troughs (60 ml and 300 ml) • Freedom EVOware® Standard software package
Equipment MACHEREY-NAGEL	• NucleoMag® 96 Plant kit
Third party equipment	• BioShake 3000 elm, Q.instruments • Magnetic separator VP 771G-4A, V&P Scientific • 384-well plates (deep well), Greiner BioOne; Cat. No. 781270 • 96-well plates

Table 1 Overview of equipment for ultra-high throughput gDNA extraction from plant material.

Manual steps

It is recommended that 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 a 96-well format.

Automated workflow

Sample lysates are transferred from 96-well plates into a 384-well plate for processing. The fully automated DNA extraction procedure includes binding of the DNA to the NucleoMag Plant beads, wash steps, and the final elution of the purified DNA in 20 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.

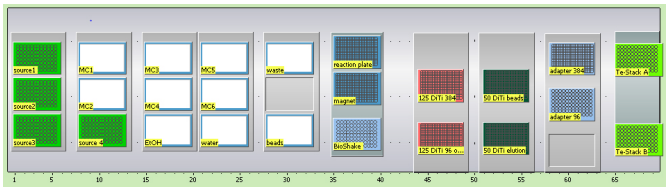

Figure 1 Freedom EVO worktable for gDNA extraction from plant material with a passive magnetic separator, shaker and Te-Stack modules.

Results

Automation of the NucleoMag 96 Plant kit on the Tecan Freedom EVO sample preparation workstation allows fast, convenient and reliable purification of plant genomic DNA. The automated extraction of genomic DNA from plant material such as maize leaves takes approximately 50 minutes for 384 samples. Automated DNA extraction on the Freedom EVO workstation and the manual method are highly comparable with regards to yield and purity.

Purity

The purity of DNA extracted with the MACHEREY-NAGEL NucleoMag 96 Plant kit is excellent (Figure 2). An average $A_{260/280}$ ratio of 1.9 was obtained with fresh maize leaf samples (Figure 3). The eluted DNA is highly pure and free of contaminants, allowing a broad range of downstream applications.

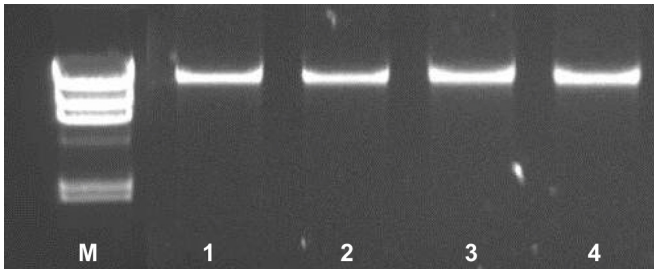

Figure 2 Extracted gDNA is highly pure. Four randomly selected gDNA samples purified from fresh maize leaves were analyzed by gel electrophoresis on a 1 % TAE-agarose gel. All samples show a high molecular weight band with little degradation (1-4), M: Lambda DNA/HindIII Marker, 2 (Thermo Scientific).

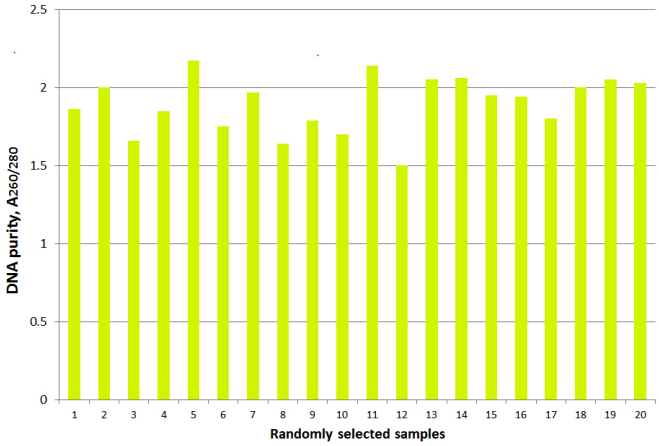

Figure 3 gDNA isolated from fresh maize leaves showed excellent purity. DNA was purified from 384 samples and 20 samples were selected randomly for analysis. Each bar represents the A260/280 ratio from a 7.5 mg sample of fresh maize leaves

Yield and reliability

Assay reproducibility is shown in Figure 4. A pool of plant lysates from frozen maize leaves was used to perform 288 extractions from identical aliquots, and gave a DNA yield of ~40 ng from a 7.5 mg sample with a CV of 7.7 %. The data highlights the robustness and consistency of the automated procedure. Typical yield from 7.5 mg of fresh maize leaves is approximately 110 ng (data not shown).

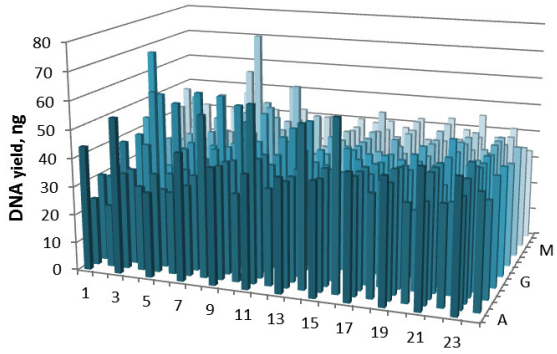

Figure 4 Reproducibility of the DNA purification process. DNA was purified from 288 samples of 7.5 mg frozen maize leaves. An average DNA yield of 39 ng was obtained. The CV of 7.7 % demonstrates the high reproducibility of the purification process.

Downstream applications and cross-contamination

A PCR-based method was chosen to demonstrate the quality of the gDNA purified by the automated process. To demonstrate the absence of cross-contamination, 288 plant lysate samples, plus PBS buffer as negative controls, were arranged in a 384-well plate in a checkerboard pattern. DNA isolation of both positive and negative samples was performed using the automated NucleoMag 96 Plant kit protocol, and 2 µl aliquots of the eluates were subjected to real-time qPCR targeting a housekeeping gene, tRNA for leucine. The results are illustrated in Figure 5. The desired PCR product was amplified in all samples (green). No amplification could be detected in the negative samples (red), indicating that there was no cross-contamination during this experiment. The purified DNA is therefore suitable for a broad range of downstream applications, including PCR and real-time PCR.

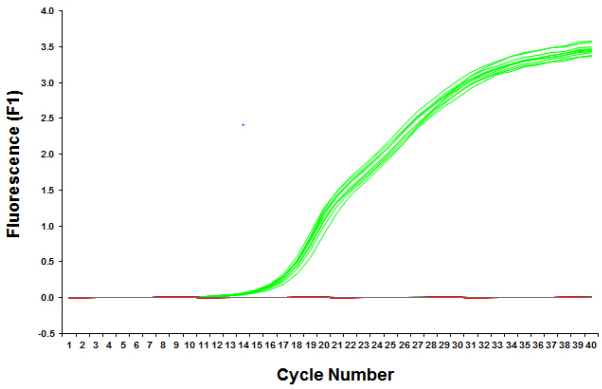

Figure 5 Cross-contamination analysis. 2 µl of gDNA eluate were amplified by PCR (Roche LightCycler™ System, tRNA leucine fragment, SYBR® Green detection, 40 cycles). Specific PCR products were amplified only from the wells which were filled with plant lysate samples (green curves). No specific PCR product was obtained from the wells filled with PBS buffer (red lines), and no cross-contamination was observed.

Conclusion

The NucleoMag 96 Plant kit combined with the Tecan Freedom EVO sample preparation workstation with a MCA 384 is an excellent solution for very high throughput DNA extraction from plant material in a 384-well format. Automation enables fast, reliable extraction of gDNA from plant material in a true walkaway manner, consistently generating high quality genetic material. 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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