Automated Purification of Plant Genomic DNA

The MACHEREY-NAGEL NucleoSpin®96 Plant Kit on a Freedom EVO Nucleic Acid Sample Preparation Workstation

Introduction

High-throughput plant DNA isolation technology plays a pivotal role in large plant genotyping projects (population studies, plant breeding investigations and genetic modification of organisms).

However, the throughput, quality and quantity of total DNA prepared are often the limiting steps for downstream genetic analysis.

Flexible workstations. Tecan offers different solutions on the Freedom EVO platform for genomic applications using vacuum filtration/solid phase extraction, magnetic bead separation or centrifugation. Each system addresses the needs for higher throughput DNA processing while at the same time delivering the highest possible performance. This publication shows how the Tecan Freedom EVO platform can be readily used for the automated purification of plant genomic DNA.

Automated solutions. The Tecan Freedom EVO Sample Preparation Workstation provides a highly flexible platform for the NucleoSpin®96 Plant Kit from MACHEREY-NAGEL. The combined use of the Tecan vacuum filtration option (Te-VacS) and the integrated robotic manipulator (gripper tool) allows fully automated plant DNA extraction in a 96-well format. Moreover, by using different storage modules on the Freedom EVO platform, multiple batches of 96 samples can be isolated without any manual user intervention.

The reliability of the DNA extraction and its suitability for downstream applications like conventional Polymerase Chain Reaction (PCR) and Random Amplification of Polymorphic DNA (RAPD) are demonstrated.

Materials and Methods

Plant leaves are freeze-dried for 12 hours. After weight determination, the samples are ground in a round-well block with 3-mm diameter metal beads using a Retsch MM300 homogenizer. Grinding is performed at 30 rps for 20 seconds.

DNA extraction. After plant samples are homogenized, the DNA is extracted with lysis buffers containing denaturing agents and detergents. Mixtures after lysis are cleared by vacuum to remove polysaccharides, contaminating material and residual cellular debris. The clear supernatant is mixed with binding buffer and ethanol to create conditions for optimal binding of plant DNA to the silica membrane. After washing with two different buffers, DNA is eluted in low-salt buffer or water and is ready-to-use for subsequent reactions such as PCR, RAPD, Amplified Fragment Length Polymorphism (AFLP) or Southern blotting.

PCR. Specific PCR of extracted plant DNA samples is performed using a primer pair for the TrnL Intron (Taberlet et al., 1991). Random amplification was tested with the M2 RAPD Primer (5'-ACA ACG CCT C-3') with 1 to 5 µl of extracted DNA as template.

Automation. Automation of the plant DNA extraction method using the MACHEREY-NAGEL NucleoSpin®96 Plant Kit is performed on a Tecan Freedom Sample Preparation Workstation. Ground plant samples and reagents from the MACHEREY-NAGEL NucleoSpin®96 Plant Kit are placed on the deck of the platform (refer to Fig.1) and the automated process is started. The instrument is equipped with 8 pipetting channels (disposable tips) and a robotic manipulator arm. The integrated Te-VacS module performs the vacuum filtration steps. Gemini software is used to control of the process. Final elution volume was 100 or 200 µl.

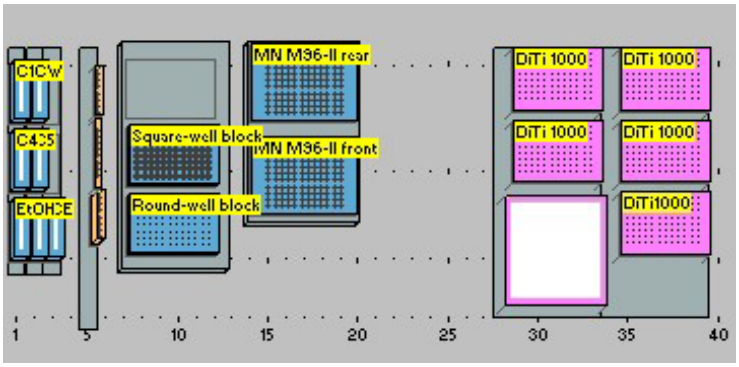

Fig. 1: Worktable layout
Full automation of the NucleoSpin®96 Plant Kit from MACHEREY-NAGEL was performed on a Tecan Freedom Sample Preparation Workstation using a Te-VacS vacuum separator. Samples, reagents and consumables for multiple batches can be placed on the instruments worktable.

Results

Fast and reliable DNA extraction was achieved using ground freeze-dried leaves from various plant species. Sample sizes ranged from 5 mg (for amplification purposes) up to 75 mg (preparative run; Fig.2). For all samples, extraction yield was consistently within the range of 120-420 ng DNA per mg dried plant tissue. Purified DNA was free of PCR-inhibiting contaminants as shown by accurate and homogeneous amplification results (Fig. 3; Fig. 4).

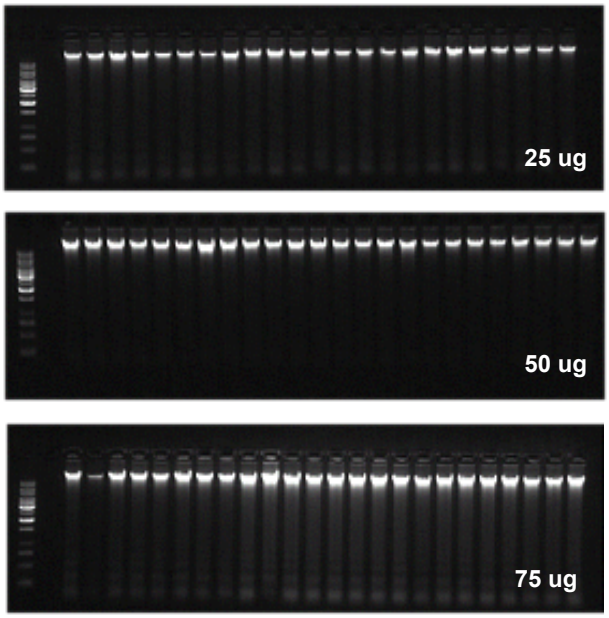

Fig 2: DNA extraction from different amounts of leaf material
Leaves were lyophilized prior to extraction and homogenized in a pool to show well-to-well consistency of the NucleoSpin® 96 Plant Kit. DNA was isolated from the indicated amounts of wheat leaves. Elution was performed in 2 x 100 µl (recovered volume 170 µl). 15 µl purified DNA was analyzed by gel electrophoresis on a 1 % agarose gel (Tris/Acetate/EDTA buffer with ethidium bromide stain; M: 1 kB ladder(MBI Fermentas)).

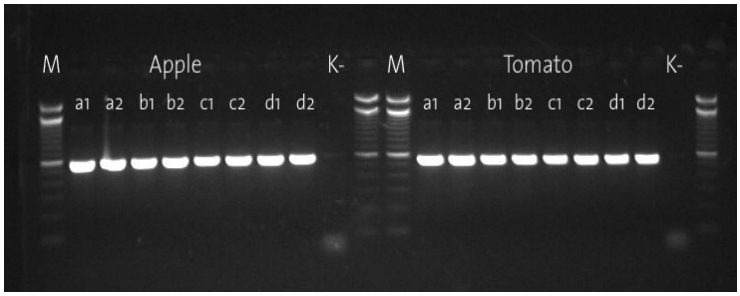

Fig. 3: PCR of extracted plant DNA
Plant DNA from apple and tomato was amplified with the primer pair for the TrnL Intron (Taberlet et al., 1991). 1 µl of DNA from different individual extractions was used as template in 50 µl reaction volume. 7 µl of the PCR reaction were loaded on a 2% agarose gel in 1 x Tris/Borate/EDTA (TBE) and were run at 90V for 3.5 h.
(M = Marker; a, b, c, d = Samples, duplicate amplification; K=Control)

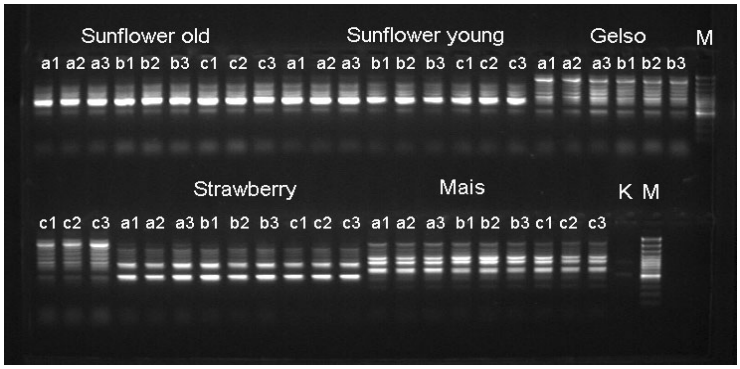

Fig. 4: RAPD using extracted plant DNA
DNA from different plant species was amplified using a RAPD Primer M2 (5'-ACA ACG CCT C-3'). 5 µl of DNA from different individual extractions was used as template in 15 µl reaction volume. 7 µl of the PCR reaction were loaded on a 2% agarose gel in 1 x TBE and were run at 90V for 3.5 h. (M = Marker; a, b, c, d = samples, triplicate amplification; K=Control).

Conclusion

The NucleoSpin®96 Plant Kit from MACHEREY-NAGEL automated on a Tecan Freedom EVO Nucleic Acid Sample Preparation Workstation guarantees reliable plant DNA extraction with excellent results:

- 96 samples are processed in about 150 minutes
- homogeneous yields from a broad range of plant species.
- DNA is ready-to-use for downstream applications like PCR and Southern blotting.

For the highest possible flexibility, the open platform concept of the Freedom EVO Nucleic Acid Sample Preparation Workstation provides significant process extension possibilities. This includes straightforward integration of an absorbance reader, thermocycler and additional cooling, heating or shaking devices.

References

Taberlet P, Gielly L, Pautou G & Bouvet J (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology*, ***17***, *1105–1109.*

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