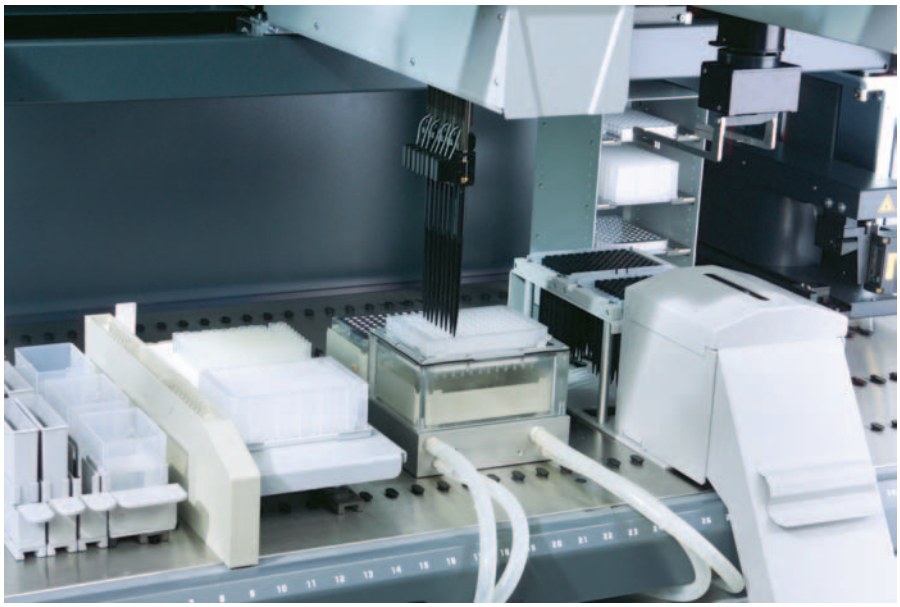
Automated DNA Extraction from Plants

High-throughput purification of plant DNA on a Tecan Freedom EVO™ Nucleic Acid Sample Preparation workstation



A high-throughput plant DNA isolation technology has been established to support projects that help to find better solutions for the control of plant diseases.

Research on plant diseases

Downy mildew, caused by the oomycete *Plasmopara viticola*, is one of the most important grape *(Vitis vinifera)* diseases, which needs to be controlled by preventive fungicide application to reduce its erratic appearance and high reproduction potential. One of the research areas of the group of Cesare Gessler at the Institute of Plant Sciences of the Swiss Federal Institute of Technology in Zürich is to investigate the mechanisms and to find better solutions for the control of this disease. Four microsatellite markers have been developed to clarify unknown aspects of epidemiology and genetic variability of the pathogen.

A high-throughput plant DNA isolation technology has been established to support the projects in this laboratory. The automation of this method plays a pivotal role in many large plant genotyping projects, such as population studies, plant breeding, GMO investigations and many others. However, throughput, quality and quantity of total DNA prepared are often the limiting steps for downstream genetic analysis.

The Tecan Freedom EVO Nucleic Acid Sample Preparation workstation is a highly flexible platform for fully automated plant DNA extraction in a 96-well format. Moreover, with the use of appropriate storage modules within the Freedom EVO Nucleic Acid Sample Preparation workstation, multiple batches of 96 samples can be isolated without any manual user interaction.

Extraction principle

After the plant samples have been homogenized DNA can be extracted through lysis buffers containing denaturing agents and detergents. Lysis mixtures are cleared by centrifugation in order to remove residual cellular debris. The clear supernatant is mixed with binding buffer containing chaotropic salts and ethanol to create conditions for optimal binding to the silica membrane. After washing with two different buffers, DNA can be eluted in low salt buffer or water and is ready-to-use for subsequent reactions such as PCR, RAPD, AFLP or Southern blotting.

Automation

The Tecan Freedom EVO Nucleic Acid Sample Preparation workstation is designed for the automation of plant DNA extraction with kits such as the Macherey-Nagel NucleoSpin® 96 Plant. The instrument is equipped with 8 pipetting channels for disposable tips and a robotic manipulator (RoMa) arm. The Gemini software package is used for control of the process, and all steps requiring vacuum conditions are performed using the integrated Te-VacS module.

The complete automated process is divided into three steps. Lysis of the material in the incubator for 30 minutes, centrifugation for 20 minutes and finally the cleaning up performed by Freedom EVO with Vacuum pumps, taking 70 minutes for 96 samples. This adds up to a total processing time of 2 hours for 96 samples.

The process

Plant leaves were freeze-dried for 12 hours and, after weight determination, were ground in a Round-well Block with

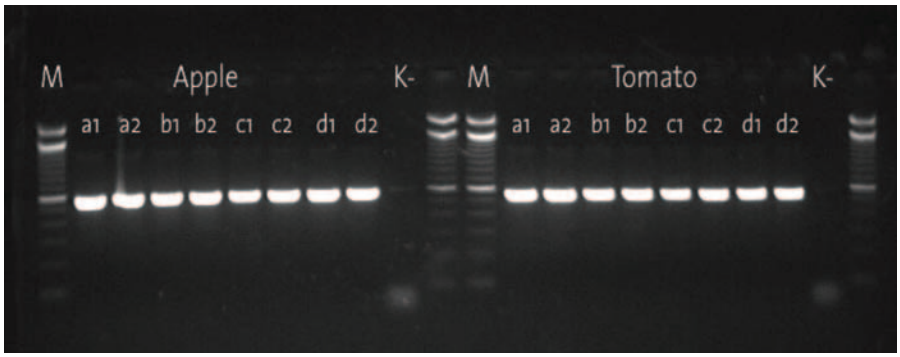


Figure 2: PCR from extracted Plant DNA. Plant DNA from different plant species was amplified with the primer pair for the TrnL Intron (Taberlet et al., Plant Mol Biol. 1991 (5):1105-9). 1 µl of DNA from different individual extractions was used as template in 50 µl reaction volume. 7 µl of the PCR reaction were loaded on a 2% agarose gel in 1x TBE and were run at 90V for 3.5h. Similar results demonstrating the quality and reproducibility of the automated DNA extraction were also received for the following plant species: sunflower, strawberry and maize (not shown). (M = Marker; a, b, c, d =Sample, duplicate amplification; K=Control).

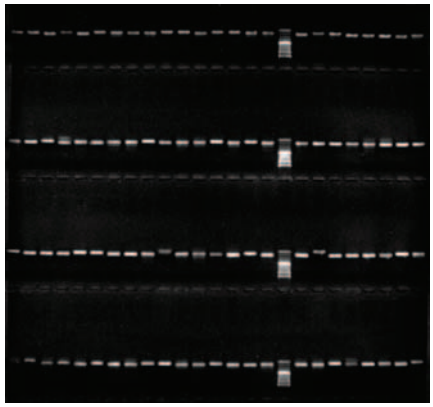


Figure. 3 HTP extraction of DNA from Plasmopara viticola. *The obligate biotrophic parasitic oomycete* Plasmopara viticola *is the causal agent of grapevine downy mildew.* P. viticola *DNA was co-extracted with* Vitis vinifera *DNA from infected leaves (96 samples). DNA extraction was performed using NucleoSpin® 96 Plant on a Freedom EVO Nucleic Acid Sample Preparation workstation. After PCR amplification with the SSR marker CES specific to* Plasmopara viticola *DNA, the fragments were loaded onto a 1% agarose gel.*

3mm metal beads in a Retsch MM300 mixer mill. Grinding was performed at 30 s^{-1} for 20 seconds.

Ground plant samples and reagents from the NucleoSpin® 96 Plant kit were placed on the deck of the Freedom EVO Nucleic Acid Sample Preparation workstation and the automated process was started. The final elution volume was 100 µl.

Specific Polymerase Chain Reactions of extracted plant DNA samples were performed using a primer pair for the TrnL Intron (Taberlet *et al*., Plant Mol Biol. 1991 (5):1105-9). 1 to 5 µl of extracted DNA was used as a template. Parasitic specific amplification (Fig. 3) was achieved with the SSR marker CES specific to *Plasmopara viticola* DNA.

Results

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

Conclusion

The Freedom EVO Nucleic Acid Sample Preparation workstation in conjunction with the NucleoSpin®96 Plant kit guarantees reliable plant DNA extraction with excellent results:

- 96 samples are processed in about 120 minutes.
- Homogeneous yields from a broad range of plant species.
- DNA is ready-to-use for downstream applications including PCR, Southern Blot and customer specific applications such as analysis of co-extracted parasite DNA.

Data kindly provided by G. Valsesia and D. Gobbin of the group of Cesare Gessler at the Institute of Plant Sciences, Swiss Federal Institute of Technology, Zürich, Switzerland.