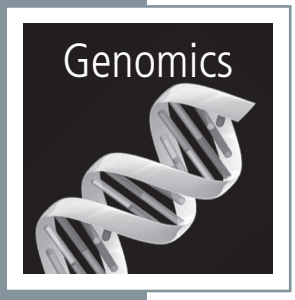

Automated Genomic DNA Extraction from Grapevine

DNA extraction from plants is a very demanding procedure. Numerous secondary metabolites such as polyphenolics, polysaccharides or acidic components can contaminate the sample and require several time-consuming processing steps. Especially for reproducible PCR results, a protocol yielding high quality DNA in a sufficient quantity is required. Here, we present an automated and efficient method for genomic DNA extraction from grapevine implemented on the Microlab® STAR liquid handling robot using the NucleoSpin® 8 Plant kit from MACHEREY-NAGEL and assess the quality of the extracted DNA.

Features & Benefits

- **Flexible sample number**

  The MACHEREY-NAGEL 8-well strips can be flexibly loaded to match your sample number

- **Short hands-on time**

  The method is fully automated from clarified samples to eluted DNA and takes less than one hour for up to 48 samples

- **Reliable Results**

  The extraction yields high quality DNA suitable for PCR and other down-stream applications

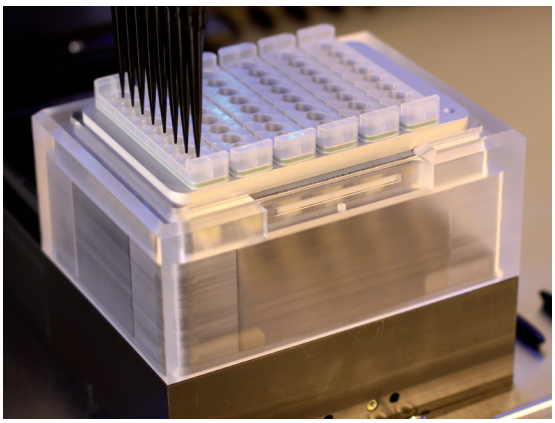

Figure 1: *Microlab STAR Basic Vacuum System (BVS) loaded with MACHEREY-NAGEL 8-well strips.*

Protocol

Deck layout

The deck is equipped with the Microlab® STAR BVS (Basic Vacuum System) for automation of all filtration steps (Figure 1). Assembly and disassembly of the vacuum box as well as handling of the elution plate is carried out with the CO-RE gripping tool. Additional carriers are used for tips and reagents.

Application Software

The method was developed with the Microlab® VENUS one software. All labware definitions, liquid classes and control of the vacuum system are included in the method.

Kit Description

The MACHEREY-NAGEL NucleoSpin® 8 Plant kit can be used for the extraction of genomic DNA from plant tissue based on vacuum filtration. After homogenization, the DNA is extracted with CTAB buffer and the crude lysate is cleared by centrifugation. The cleared supernatant is mixed with binding buffer and ethanol to create optimal conditions for the DNA to bind to the silica membrane in the NucleoSpin® Plant binding module on the vacuum system. Two wash cycles with different buffers follow until the DNA is finally eluted using low salt buffer or water, ready to use for subsequent reactions.

Method

Samples were manually prepared prior to loading onto the Microlab® STAR as follows: About 100 mg of young grape leaves, 400 µL Buffer C1, 10 µL RNase A and one steel bead were collected in a 2 ml reaction tube. Samples were homogenized in the TissueLyser II from Qiagen for 1min at 28Hz, centrifuged for 60 s at 3000 x g and incubated at 56°C for 30 min. After centrifugation at 6000 x g for 20 min the clarified samples were transferred

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to MACHEREY-NAGEL 8-well strips on the Microlab® STAR and further processed according to the MACHEREY-NAGEL NucleoSpin® 8 Plant protocol.

Results

Genomic DNA was extracted from different Vitis cultivars (Grenache noir and Chardonnay) and rootstock (101.14) and quality and quantity were analyzed. The amount of DNA was measured with the Quant-iT Broad-Range DNA Assay kit (Invitrogen). In order to verify the varietal identity, a multiplex PCR with three microsatellite loci, which have been selected by the Genres 081 European Project for differentiation of grapevine cultivars (VVS2, VVMD7 and VrZAG62) was performed (Figure 2).

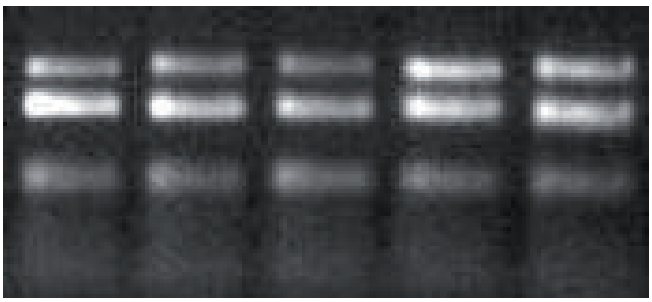

Figure 2: *Multiplex PCR of three microsatellite loci; lane 1 to 5, amplicons from different Vitis cultivars and rootstock 101.14*

The samples were further characterized by PCR with Cy5 labelled primers (VMC6E1, VrZAG62 and VVMD7) using 10ul of genomic DNA, 10µl Taq-dNTPs Mix (Eppendorf), 0.25 µl forward and 0.25 µl reverse SSR primers (20 pmol/µl). The microsatellite PCR fragments were visualized by electrophoresis using the ALFexpress-II sequencer (Amersham, Figure 3).

Figure 3: *ALFexpress-II analysis of microsatellite PCR reaction; lane 1 Grenache noir, lane 2, rootstock 101.14*

Yield and quality

About 1 ug of genomic DNA was extracted from 100mg of young leaves, comparable to manual extractions. The λ260/λ280 ratio average was 1.85.

Throughput and capacity

Up to 48 clarified samples can be processed in less than one hour without user intervention. Higher throughputs and longer walk-away times can be achieved by switching to the NucleoSpin® 96 Plant kit.

Discussion

The isolation of genomic DNA from plant materials can be reliably automated on the Hamilton STAR with the MACHEREY-NAGEL NucleoSpin® 8 Plant kit. The MACHEREY-NAGEL 8-well strips allow an adjustment of the throughput of the system to the actual workload and to process up to 48 samples per run.

The isolated DNA is ready for downstream applications, which can be implemented on the same platform taking advantage of Hamilton's highly flexible and modular pipetting workstation.

Equipment and Materials

Equipment

- Microlab® STARlet equipped with 8 independent 1000µl channels, the CO-RE grip robotic plate handler and various carriers for tubes, plates, reagents and disposable tips. Functional modules include a plate shaker (Variomag Teleshake) and the Microlab® BVS vacuum system with ME 4C Vario Membrane pump and CVC 2000 Controller (Vacuubrand GmbH, Wertheim, Germany)
- Starter Set A containing a column holder and dummy strips (MACHEREY-NAGEL GmbH, Düren, Germany)

Reagents

- NucleoSpin® 8 Plant kit (MACHEREY-NAGEL GmbH, Düren, Germany)

Acknowledgements

We would like to thank Dr. Stefano Meneghetti, Dr. Enrica Frare and Dr. Manna Crespan from CRA-VIT, Research Center for Viticulture, Susegana (Tv), Italy for providing all experimental data.

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