APPLICATION NOTE

Automated DNA Extraction from different samples (grapevine roots, wheat and olive leaves) using NucleoMag® Plant kit and OMNIA *Prima* by MASMEC Biomed

Introduction

The extraction of nucleic acids (DNA, RNA, microRNA, etc.) from various biological samples represents a fundamental step for the genetic and biology molecular analysis.

This phase is often a bottleneck for the overall duration of the DNA analysis operations; moreover the quality of the data, in terms of yield, purity and absence of contamination, is affected by the variables related to the operator's manual skills.

To meet these needs, MASMEC Biomed designed and produced OMNIA *Prima*, the fully integrated workstation that automates the process of DNA extraction using the magnetic beads technology of NucleoMag® kits by MACHEREY-NAGEL. These kits allow the extraction of nucleic acids (for yield and purity) suitable for downstream applications.

The automated walk away process allows to obtain DNA/RNA in optimal quantity and quality for subsequent applications, in a short time and starting with several kind of sample material. The freely configurable worktable and the simple and intuitive management software enable high flexibility and efficient control process.

Equipments, materials and protocols

Workstation: OMNIA *Prima* configured with 1 high precision dispensing channel for liquid handling (1-1000ul) and level sensor, a magnetic tool with 12 rods to allow the attraction of the beads dispensed in plate, a thermoshaker with integrated adapter to perform the thermal and mechanical lysis of the sample.

Reagents: NucleoMag® Plant (from MACHEREY-NAGEL GmbH, Dueren, Germany)

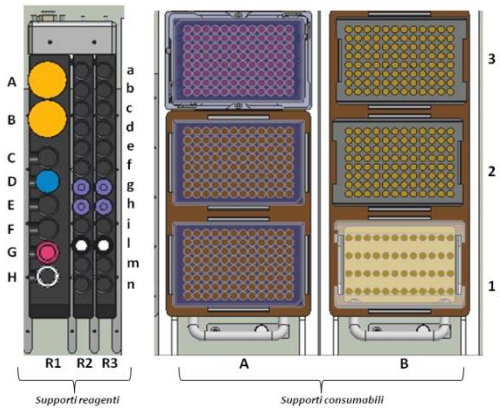

Figure 1. Example of OMNIA *Prima* internal layout

Figure 2. Vine branch and olive leaf were used as starting material

Consumables: 1 deepwell plate for lysis and elution, 1 deepwell plate for washing steps, 50, 200 and 1000ul filtered tips, 15-50 ml tubes.

Automated Protocol:

- Addition of buffer MC1
- Addition of C-Beads and buffer MC2
- incubation at 56°C for 30 min with shaking (1100 rpm)
- Dispensing of washing solution (in other plate)
- Dispensing of elution solution
- Catching of beads by magnetic tool
- Up-down washing steps
- Up-down elution step

The procedure involves the lysis of the sample (after external homogenization) starting with enzymatic and mechanical action to facilitate the breakdown of biological membranes and access to the genetic material contained in the individual cells. Particular magnetic beads bind DNA in a reversible way and then release it in elution solution after a

series of serial and stringent washes. The manual action of the operator is limited in this way to initial homogenization of sample (using liquid nitrogen or other methods).

***Software*:** OMNIA Prima is managed by the Framework software thanks to which it is possible to configure the layout of the instrument, edit customizable scripts, set parameters such as the number of incoming samples, the time and heat of the thermoshaker, all pipettable volumes, number of washes, magnetic catch times, elution volumes, etc.

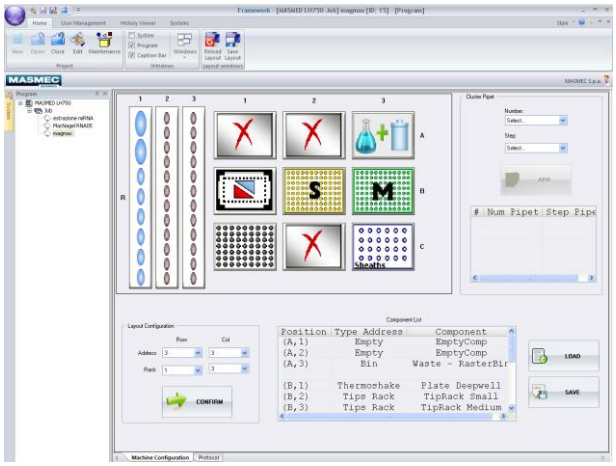

Figure 3. Screenshot of Framework software

Results

Thanks to the automation of the NucleoMag® Plant protocol with the OMNIA *Prima* workstation produced by MASMEC Biomed, DNA extracted from different agro-food matrices in *walk-away* mode can be obtained (12 samples at the same time), freeing up the operator from repetitive tasks reducing pipetting errors and the use of toxic substances in total absence of cross-contamination intra-assay and inter-assay. All the tests were conducted comparing yield and purity with manual procedures obtaining comparable data.

***Yield and quality*:** Through electrophoretic run on agarose precast gel (1,2%), the quality of the high molecular weight genomic DNA (starting from grapevine roots samples, *vitis vinifera*) with total absence of degradation was assessed (Figure 4).

The figure 5 shows the comparison between average of DNA yields obtained with the NucleoMag® Plant kit by extracting DNA with manual procedures and with OMNIA *Prima* workstation and starting from grapevine roots (*Vitis vinifera*) samples. The automatic extraction allowed to obtain a superior yield respect to the manual.

NucleoMag® Plant kit in association with OMNIA *Prima* was used also for DNA extraction from wheat to establish different genomic varieties (Figure 6) and from olive leaves (Figure 7).

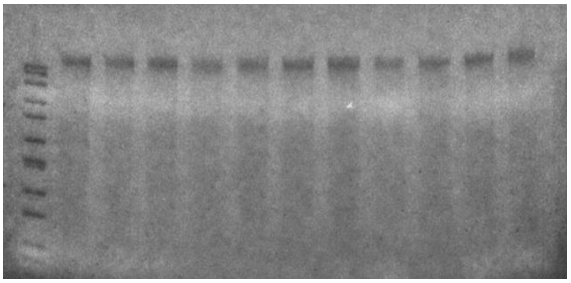

Figure 4. Genomic DNA quality observed after electrophoretic run on 1,2% E-Gel

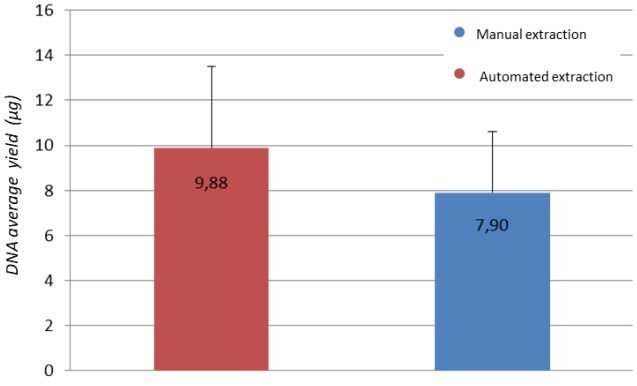

Figure 5. Average of DNA extracted from vine branches

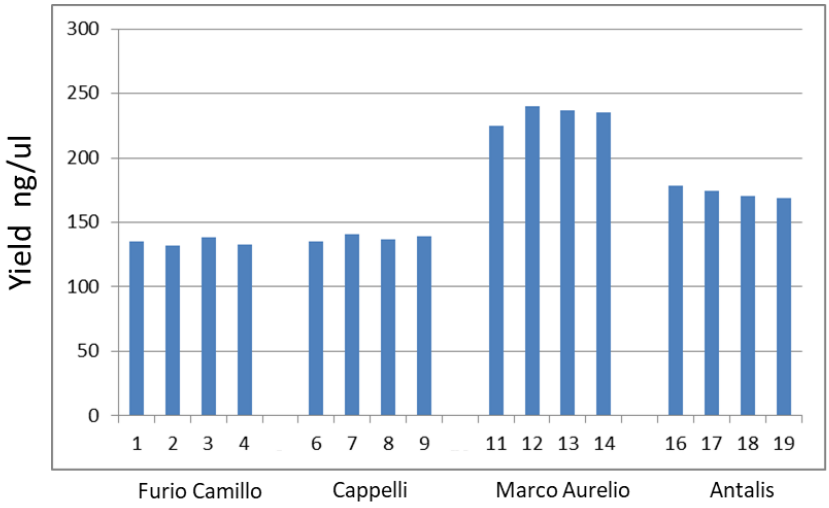

Figure 6. Genomic DNA extracted from 4 different **wheat varities** (50mg of homogenate)

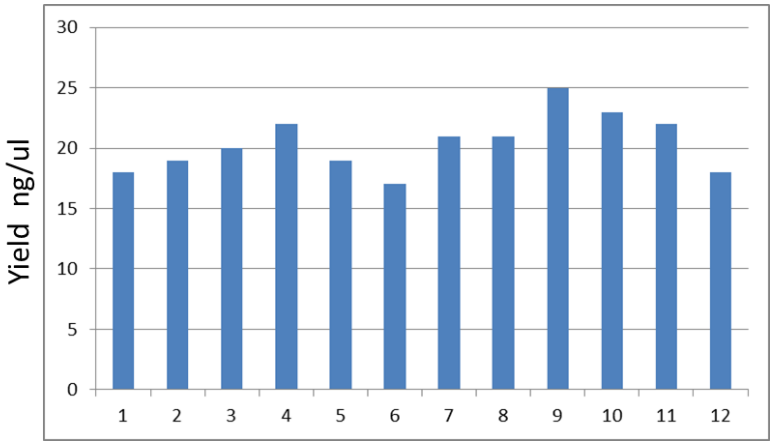

Figure 7. Genomic DNA extracted from **olive leaves** (50 mg of homogenate)

Downstream analysis:

In order to further test the quality of the extracted DNA and demonstrate the absence of inhibitors and contaminants, chloroplast DNA amplification tests were performed by PCR. Universal primers have been used that amplify the intron of the trnL gene. Moreover Real Time PCR analysis were performed to evaluate the integrity and usability of the extracted DNA (primers designed for the GAPDH gene, Figure 9) starting from grapevine.

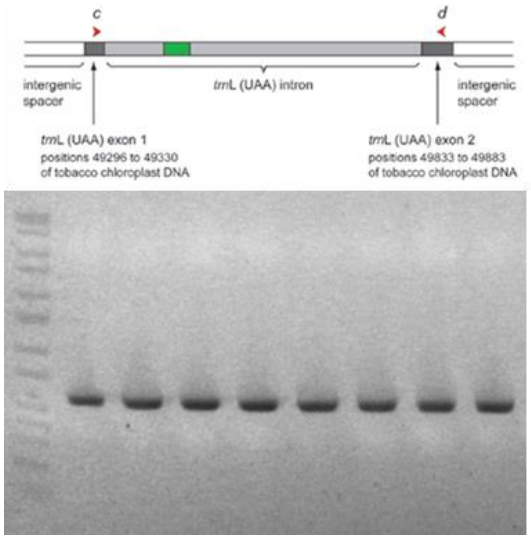

Figure 8. PCR products of chloroplast DNA. The amplicon is 600bp

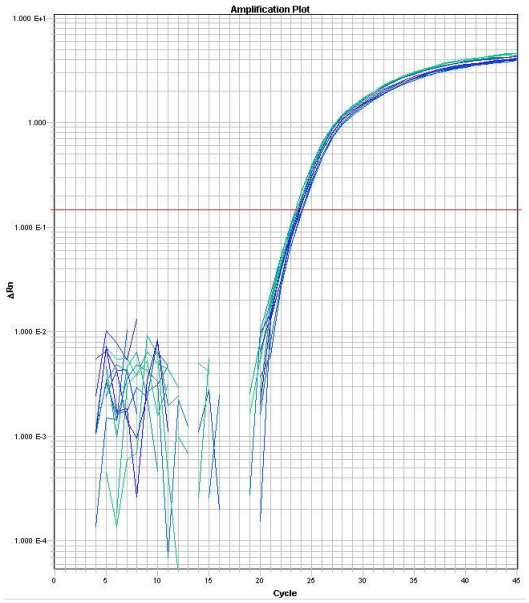

Figure 9. GAPDH amplification curves for 12 DNA samples extracted from grapewine

Conclusions

With OMNIA *Prima* is possible to perform automatic extraction of DNA from different agro food matrices using NucleoMag® Plant. The conducted experiments show yields, purities and qualities comparable or superior to manual operations. In a walk-away mode, the user is only required to homogenate samples with liquid nitrogen or other methods and to load the reagents and consumables, choose the appropriate protocol and start run. The throughput of the instrument allows to extract up to a maximum of 24 samples per run quickly and accurately.