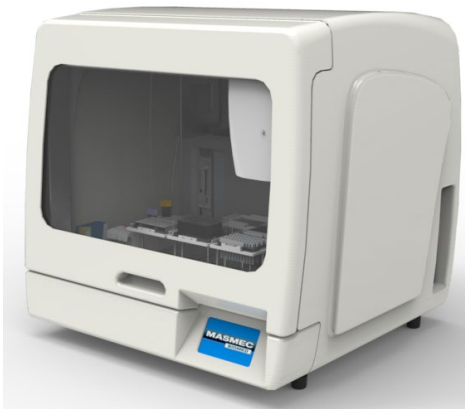


Automated “walk-away” DNA Extraction from FFPE samples using MACHERY-NAGEL NucleoMag® DNA FFPE and OMNIA by MASMEC Biomed



Introduction

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

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, the fully integrated workstation that automates the process of DNA extraction using the magnetic beads technology of NucleoMag® kits by MACHERY-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 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 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® DNA FFPE (from MACHEREY-NAGEL GmbH, Dueren, Germany)

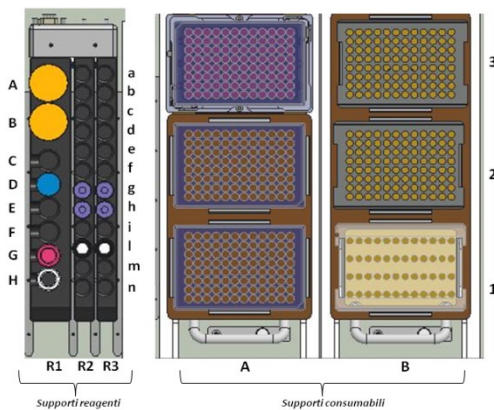


Figure 1. Example of OMNIA internal layout

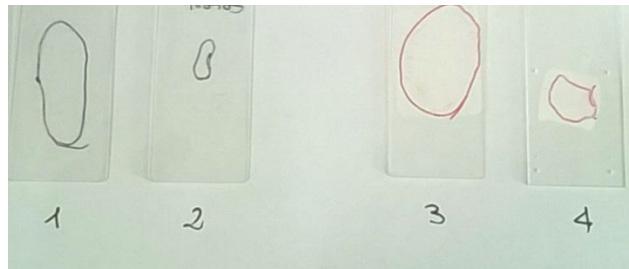


Figure 2. Example of different FFPE sections used for validation (colon, lung, brain)

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

Automated Protocol:

- Addition of Paraffin Dissolver, FL and Proteinase K (deparaffination and lysis)
- Mixing and incubation for 60 minutes at 56°C
- Addition of D-Link Buffer (decrosslinking)
- Addition of B-Beads and Buffer MB2
- 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 initial deparaffination of the sample starting with enzymatic and mechanical lysis 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 the loading of the FFPE sections directly in plate and the other consumables of deck layout.

Software: OMNIA 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.

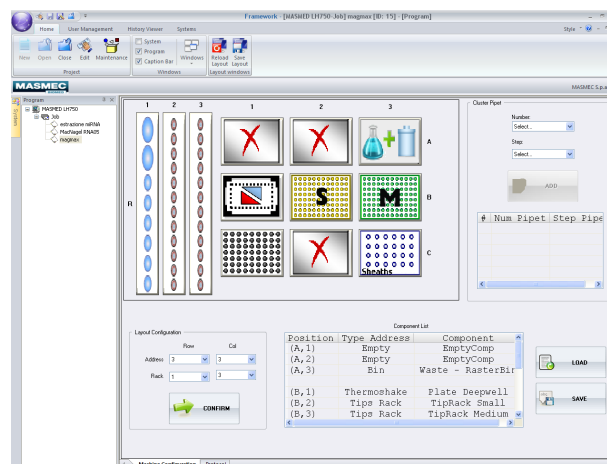


Figure 3. Screenshot of Framework software

Results

Thanks to the automation of the NucleoMag® DNA FFPE protocol with the OMNIA workstation produced by MASMEC Biomed, DNA extracted from FFPE tissue sections in walk-away mode can be obtained in just 2 hours (deparaffination included), 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 repeatability: The figure 4 shows an intra-assay reproducibility test in which the same 6 sample (colon-sections of 5 µm) was used for a complete run, obtaining an average yield of 21.64 ng/µl.

The figure 5 shows individual DNA yield comparison (n = 12) obtained with the NucleoMag® DNA FFPE kit by extracting DNA with manual procedures and with OMNIA workstation using different types of FFPE sections (colon, lung, brain). In any case, the automatic extraction allowed to obtain a comparable yield respect to the manual and respect to section dimension.

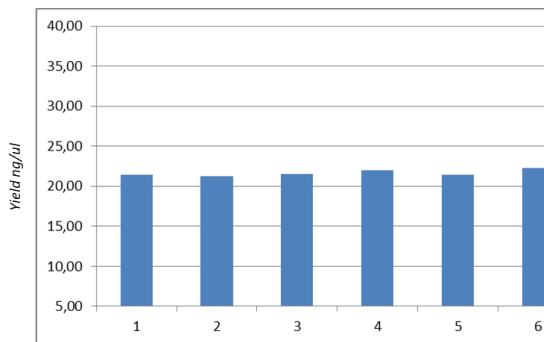


Figure 4. DNA isolation from 6 same FFPE sections (colon)

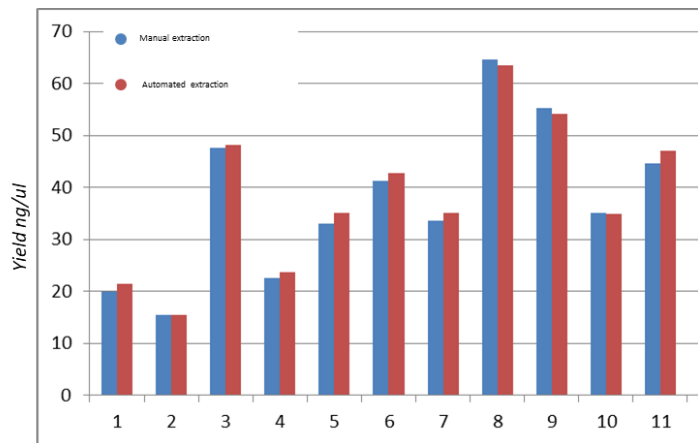


Figure 5. Individual DNA yields starting from different FFPE sections of 5 µm

Purity:

The Figures 6 and 7 show the purity measured by spectrophotometry and absorbance ratio at 260/280 nm and 260/230 nm, obtained from 12 FFPE samples extracted with OMNIA. The eluted DNA is highly pure and free of proteins and contaminants, so it can be used for downstream applications.

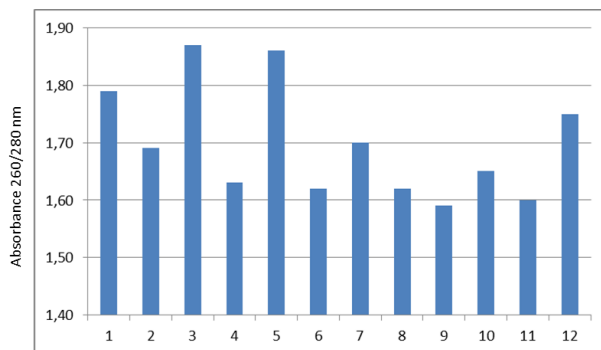


Figure 6. Purity (absorbance 260/280 nm)

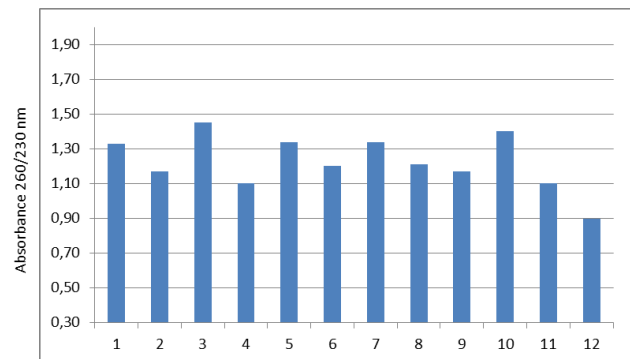


Figure 7. Purity (absorbance 260/230 nm)

Conclusions

With OMNIA is possible to perform automatic extraction of DNA from FFPE tissue using NucleoMag® DNA FFPE kit. The conducted experiments show yields and purities comparable to manual operations. In a walk-away mode, the user is only required to load sections in 96-deep well plate, 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.

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