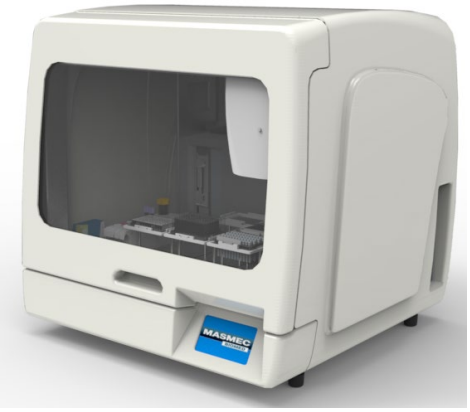
Automated *"walk-away"* Genomic DNA Extraction from blood samples using MACHEREY-NAGEL NucleoMag® Blood 200 µL kit and OMNIA by MASMEC Biomed



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 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 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® Blood 200 µL (from MACHEREY-NAGEL GmbH, Dueren, Germany)

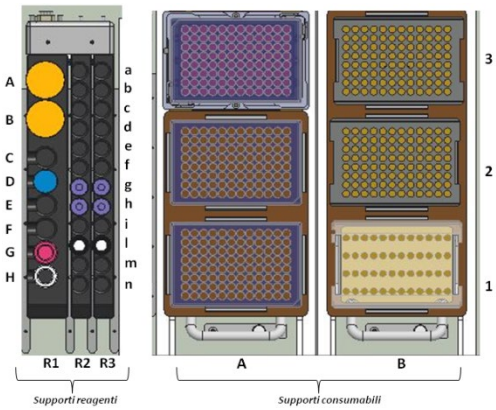


Figure 1. Example of OMNIA internal layout

Figure 2. Blood sample manual loading

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

Automated Protocol:

- Addition of lysis buffer and Proteinase K
- Addition of sample (blood, 200ul)
- incubation at 56°C for 15 min with shaking (1100 rpm)
- Dispensing of washing solution (in other plate)
- Addition of binding buffer and magnetic beads to lysed sample and shake at room temperature for 5 min
- Dispensing of elution solution
- Catching of beads by magnetic tool
- Up-down washing steps
- Up-down elution step

The procedure involves the initial disintegration of the sample (blood, 200 µl) 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 tubes containing the blood and the other consumables of instrument 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.

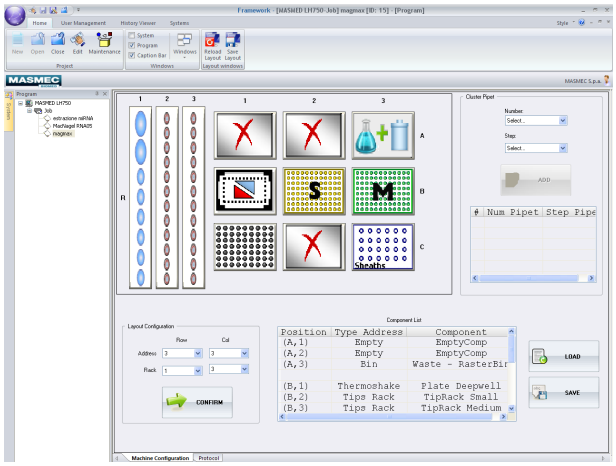


Figure 3. Screenshot of Framework software

Results

Thanks to the automation of the NucleoMag® Blood 200 µL protocol with the OMNIA workstation produced by MASMEC Biomed, DNA extracted from blood (fresh or frozen) in *walk-away* mode can be obtained in just under 40 minutes (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 repeatability*:** The figure 4 shows an intra-assay reproducibility test in which the same pool of blood was used for a complete run of 12 extractions, obtaining an average yield of 66ng / µl with a 2.8% CV.

The figure 5 shows individual DNA yields comparison (n = 17) obtained with the NucleoMag® Blood 200 µL kit by extracting DNA with manual procedures and with OMNIA workstation. In any case, the automatic extraction allowed to obtain a comparable or superior yield respect to the manual (range between 30 and 90 ng/µl).

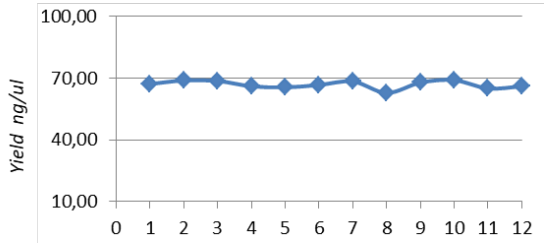


Figure 4. DNA isolation from pooled blood sample and aliquoted for 12 wells

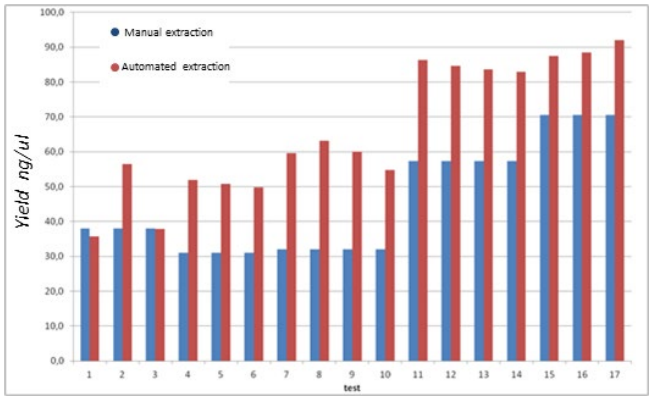


Figure 5. Individual DNA yields starting from 200ul of blood

Quality and purity:

Through electrophoretic run on agarose precast gel (1%), the quality of the high molecular weight genomic DNA with total absence of degradation was assessed (Figure 6, *DNA size standard: Lambda Hind III*). The Figure 7 shows the purity measured by spectrophotometry and absorbance ratio at 260/280 nm, obtained from different extraction runs (n = 19) with OMNIA. The eluted DNA is highly pure and free of contaminants, so it can be used for downstream applications.

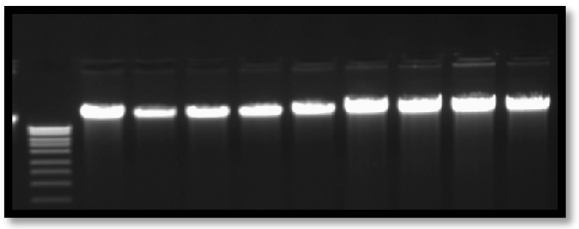


Figure 6. DNA quality observed after electrophoretic run on 1% agarose gel

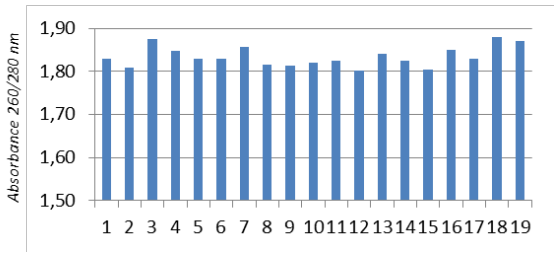


Figure 7. Purity of 19 individual gDNA extracted automatically

Conclusions

With OMNIA is possible to perform automatic extraction of DNA from blood using NucleoMag® Blood 200 µL. 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 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.