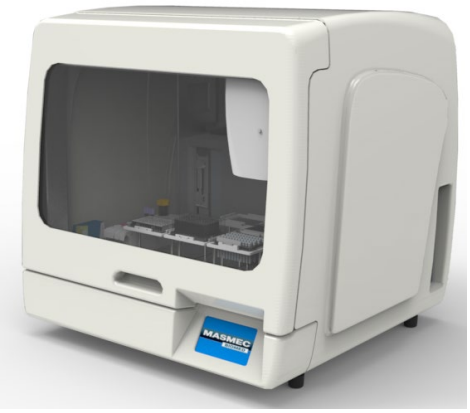
Automated *"walk-away"* total RNA Extraction from fresh and frozen tissue samples using NucleoMag® RNA kit and OMNIA by MASMEC Biomed



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/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 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® RNA (from MACHEREY-NAGEL GmbH, Dueren, Germany)

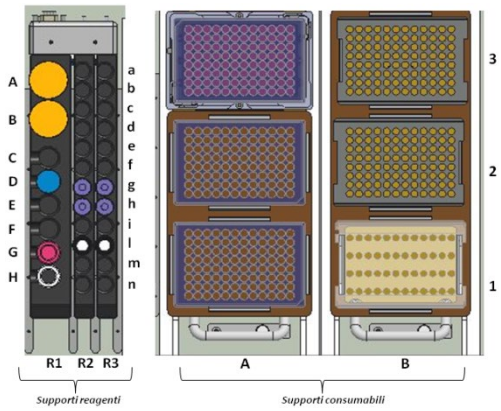


Figure 1. Example of OMNIA internal layout

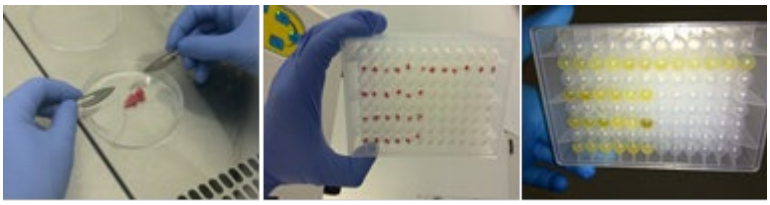


Figure 2. Manual tissue sample preparation and result of automated lysis

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 TCEP and buffer MR1 and mixing
- Addition of B-Beads and buffer MR2 and R.T. mixing (binding)
- Addition of rDNase and incubation
- Addition of MR2 and incubation (rebinding)
- 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 disintegration of the tissue sample (15 mg) 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 RNA 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 tissues 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.

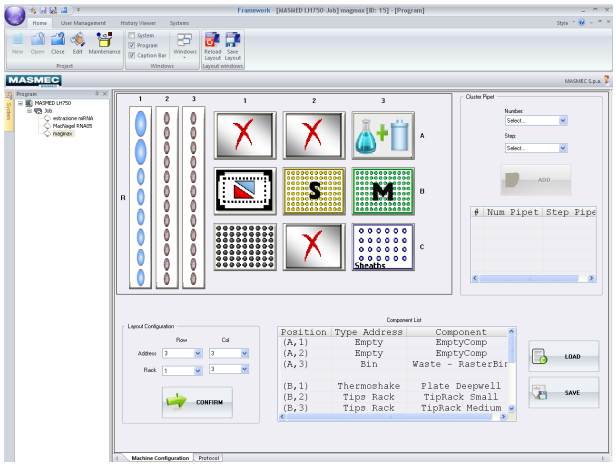


Figure 3. Screenshot of Framework software

Results

Thanks to the automation of the NucleoMag® RNA protocol with the OMNIA workstation produced by MASMEC Biomed, RNA extracted from tissue (fresh or frozen) in walk-away mode can be obtained in just 50 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 quality: The figure 4 shows an intra-assay reproducibility test in which 3 different fresh tissues was u

sed for a unique automated total RNA extraction run. Through electrophoretic run, the quality of RNA with absence of degradation was assessed (Figure 5) starting from kidney and liver tissues.

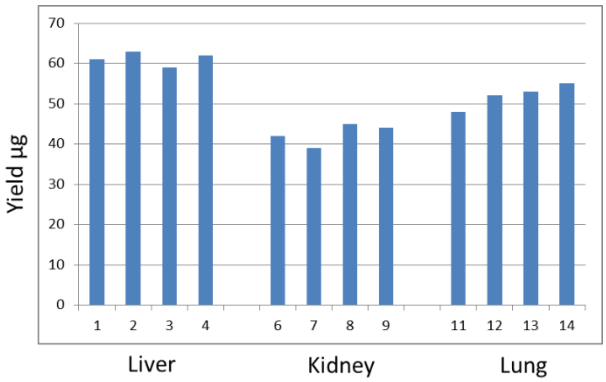


Figure 4. RNA yields of different fresh tissues after automated extraction

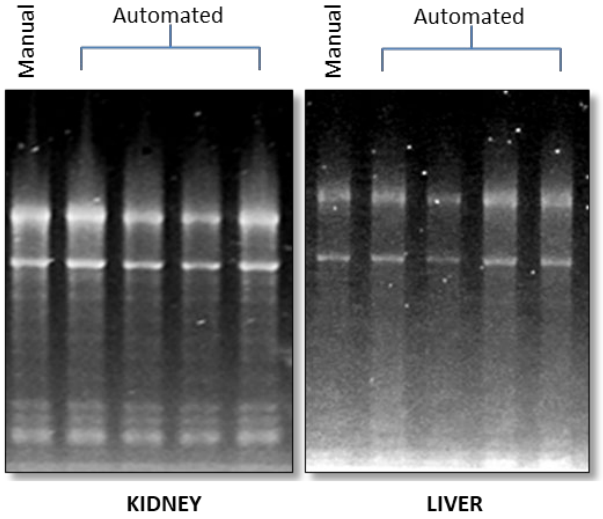


Figure 5. RNA quality observed after electrophoretic run on agarose gel

Downstream *Usability*:

The figure 6 shows the Real Time PCR amplification of β-actin (after cDNA retrotrascription) as example of downstream application after automated extraction (n = 12). The eluted RNA is highly pure and free of contaminants. Moreover integrity and usability of RNA was checked by BioAnalyzer showing a very high R.I.N. (RNA integrity number, Figure 7).

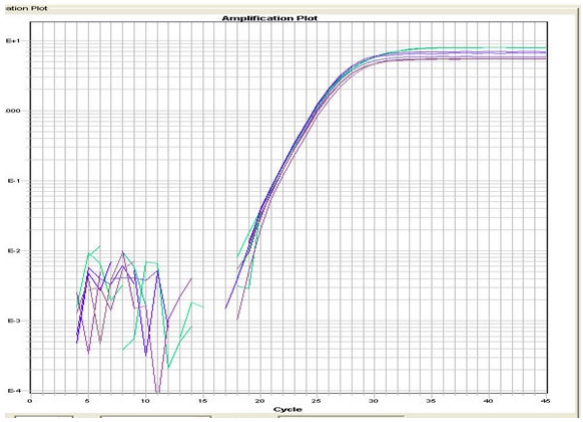


Figure 6. B-actin cDNA amplified from liver tissue (n=12)

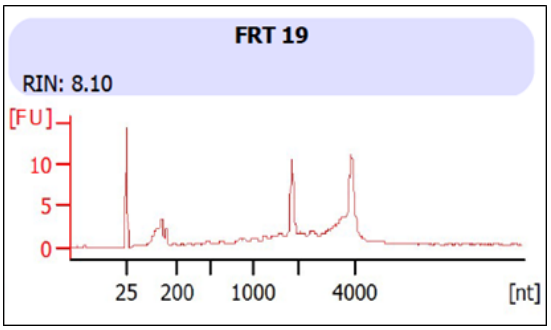


Figure 7. BioAnalyzer result of an RNA extracted with OMNIA

Conclusions

With OMNIA is possible to perform automatic extraction of RNA from fresh or frozen tissues using NucleoMag® RNA. 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.