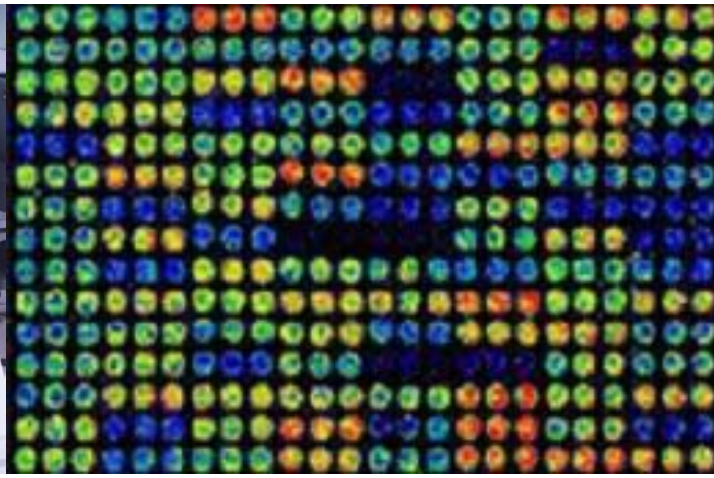
Automated RNA Extraction

The MACHEREY-NAGEL NucleoSpin® 96 RNA Kit on a Tecan Freedom Nucleic Acid Sample Preparation Workstation



Introduction

High-throughput RNA isolation technology plays a pivotal role in operation of high-throughput gene expression profiling. However, throughput, quality and quantity of total RNA prepared are often the limiting steps for downstream genetic analysis.
The Tecan Freedom Sample Preparation Workstation provides a highly flexible platform for the NucleoSpin® 96 RNA Kit from MACHEREY-NAGEL. The combined use of the Tecan vacuum filtration option (Te-VacS) and the integrated robotic manipulator (gripper tool) allows fully automated total RNA extraction in a 96-well format. Moreover, with the use of appropriate storage modules within the Sample Preparation Workstation, multiple batches of 96 samples can be isolated without any manual user interaction.
Purified RNA can be used directly for downstream applications like RT-PCR, TaqMan®, Northern/ Dot/Slot Blot and microarray analysis.
Data on the consistency of the RNA quality, reproducibility of the RNA quantity, evaluation of high-throughput RNA isolation free of cross-contamination as well as removal of genomic DNA contamination are presented.

Extraction Principle

After cell lysis, adsorption of nucleic acids onto a special silica membrane is performed in the presence of chaotropic salts. Digestion of genomic DNA with DNase I is performed directly on the silica membrane after an effective desalting for 15 minutes at room temperature. After subsequent washing steps, highly pure total RNA is eluted from the silica membrane with RNase-free water. Purification of up to 80 µg of total RNA from cells or tissue can be achieved.

Automation

Automation of the total RNA extraction method is realized on a Tecan Freedom Sample Preparation Workstation. The instrument is equipped with 8 pipetting channels (disposable tips) and a robotic manipulator arm. Gemini software was used for control of the process; vacuum steps are performed by the integrated Te-VacS module.

Worktable layout

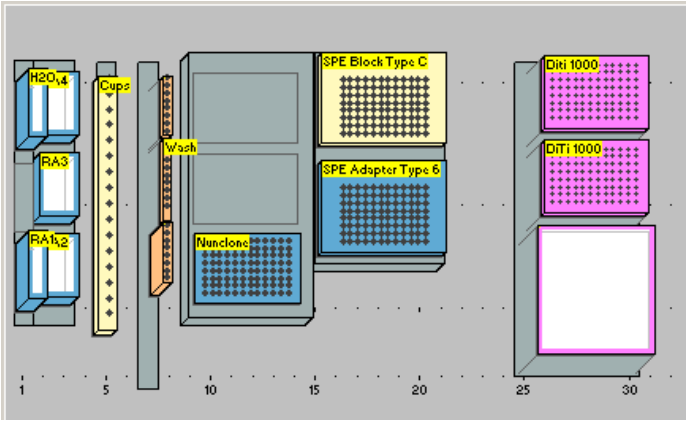


Fig. 1: Worktable layout
Full automation of the NucleoSpin® 96 RNA Kit from MACHEREY-NAGEL was performed on a Freedom Sample Preparation Workstation using a Te-VacS vacuum separator. Samples, reagents and consumables for multiple batches can be placed on the instruments worktable.
Direct RNA quantification of extracts is possible with an integrated Tecan microplate reader (not shown).

Results

High recovery of total RNA

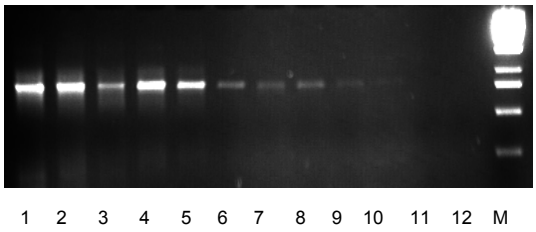


Fig. 2: High recovery of total RNA
Total RNA was prepared from the indicated amounts of liver tissue using NucleoSpin® 96 RNA and detected by RT-PCR analysis (1.5 µl out of 80 µl eluted). Limit of total RNA for RT-PCR detection: 1.5 µl/80 µl x 38 ng = 0.71 ng.
Starting material: lane 1: 6 mg; lane 2: 2.5 mg; lane 3: 0.6 mg; lane 4: 0.15 mg; lane 5: 39 µg; lane 6: 10 µg; lane 7: 2.4 µg; lane 8: 0.6 µg; lane 9: 152 ng; lane 10: 38 ng; lane 11: 7.6 ng; lane 12: 0 ng.

Reproducible yields of high-quality RNA

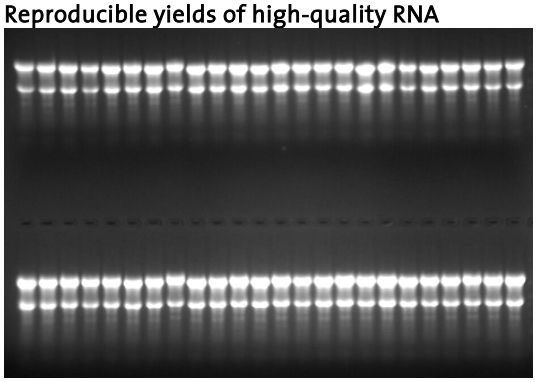


Fig. 3: Reproducible yields
Agarose gel analysis. HeLa cells (5 x 10^5) from a homogeneous cell culture were pelleted in each well of a 96-well cell-culture plate and total RNA was isolated using a NucleoSpin® 96 RNA kit on a Tecan Sample Preparation Workstation.
20 µl of each eluate (100 µl elution volume) + 5 µl 5x sample buffer were loaded and analysed on a 1.2 % formaldehyde agarose gel.
The gel shows homogeneous bands of 18S and 28S rRNA.

Removal of genomic DNA contamination

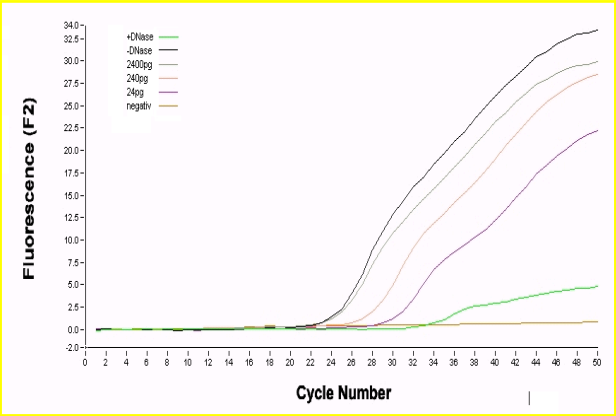


Fig. 4: PCR detection of genomic DNA
2 µl eluate of the total RNA preparation (5 x 10^5 HeLa cells) was amplified with LightCycler – DNA Amplification Kit Hybridisation Probes.
+ DNase: total RNA preparation with DNase treatment,
- DNase: total RNA preparation without DNase treatment, negative control: 2 µl water was used for PCR detection. Standard: 24 pg, 240 pg, 2400 pg genomic DNA purified from human whole blood was used for PCR detection. The remaining content of gDNA is below 0.003% .

No cross-contamination

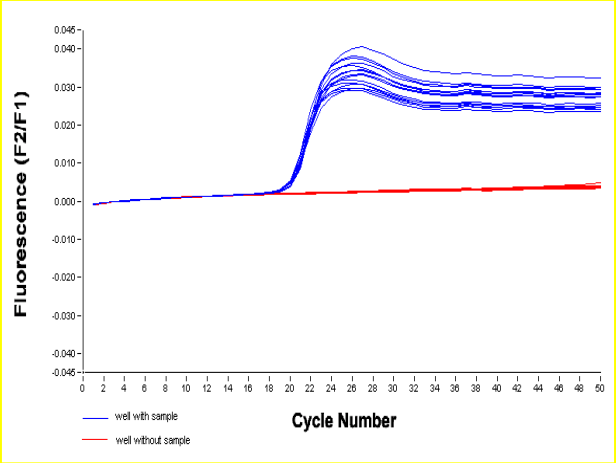


Fig. 5: RT-PCR detection of total RNA
HeLa cells (5 x 10^5 each) were pelleted in 16 wells of a 96-well culture plate in a chessboard pattern. 1.5 µl eluate of the total RNA preparation (5 x 10^5 HeLa cells) as well as 1.5 µl of the empty well eluates were amplified with Light-Cycler RNA Amplification Kit Hybridisation Probes (Roche).
50 cycles were performed using GAPDH-primer.

-------samples containing 5 x 10^5 HeLa cells

-------control samples without cells
(next to a well containing cells)

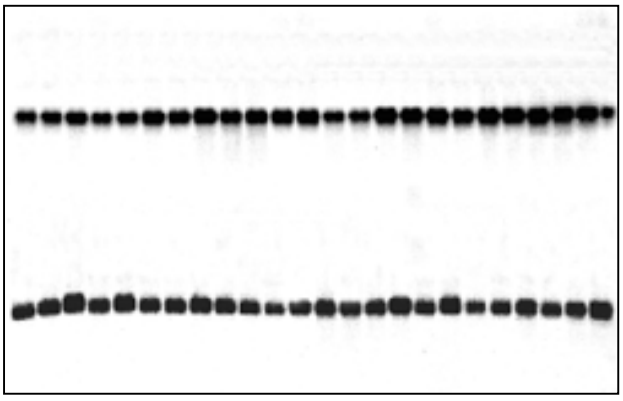


Fig. 6: Detection of gene-specific RNA by Northern blotting
HeLa cells (5 x 10^5) from a homogeneous cell culture were pelleted in each well of a 96-well cell-culture plate and total RNA was isolated using NucleoSpin® 96 RNA on a Tecan Sample Preparation Workstation.
15 µl of each eluate (100 µl elution volume) were used for a 1% denaturing agarose gel. GAPDH was used as radioactive labeled probe.

Conclusion

The NucleoSpin® 96 RNA Kit from MACHEREY-NAGEL automated on a Tecan Freedom Sample Preparation Workstation guarantees reliable RNA extraction with excellent results:

- 96 samples are processed in about 70 minutes
- Yield: maximum 20 µg of total RNA from 2 x 10^6 HeLa cells; up to 80 µg total RNA from tissue and cells (depending on starting material and effective homogenization/lysis)
- Purity: $A_{260/280}$ 1.9 - 2.1
- Very effective removal of genomic DNA: 0.003 % residual gDNA after isolation of total RNA from 5 x 10^5 HeLa cells
- Ready-to-use for downstream applications like RT-PCR, TaqMan®, Northern/Dot/ Slot Blot or microarray analysis

In addition, the open platform concept of the Freedom Sample Preparation Workstation provides straightforward process extension possibilities by the integration of an absorbance reader or additional cooling, heating or shaking devices from Tecan.

Acknowledgments

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