Genomics

# Automated Total RNA Isolation from Cells and Tissues on the Genomic STARlet

High purity and integrity of RNA are crucial for reliable gene expression studies. HAMILTON and MACHEREY-NAGEL have developed and validated the Genomic STARlet to isolate RNA from different samples as cells and tissues using the NucleoSpin® technology. This application note describes the automated isolation of RNA from Hela cells as well as from mouse liver and compares the results with a manual isolation process.

## Equipment and Materials

| System Requirements | | Part Number |
|---|---|---|
| Genomic STARlet, 4 channels, CVS Vacuum Station, HAMILTON Heater Shaker, Gene Expression Profiling Package | | 806201 |
| Genomic STARlet, 8 channels, CVS Vacuum Station, HAMILTON Heater Shaker, Gene Expression Profiling Package | | 806211 |
| **Labware Requirements, Kits** | **Size** | **Part Number** |
| NucleoSpin® 8 RNA containing 8-well strips | 12 x 8 | 740698 |
| | 60 x 8 | 740698.5 |
| NucleoSpin® 96 RNA containing 96-well plates | 2 x 96 | 740709.2 |
| | 4 x 96 | 740709.4 |
| | 24x96 | 740709.24 |

## Protocol

The deck is manually loaded with carriers containing tips, reagents, filter plates and Eppendorf tubes or micro plates with the samples. The MICROLAB® CVS Vacuum System is integrated on the deck. The plate movements as well as the loading and unloading of the vacuum manifold during the process are performed by the CO-RE Gripper (Figure 1). The Genomic STARlet is controlled by the MICROLAB® VENUS ONE software. A dedicated application interface guides the user through the instrument setup process. Further application relevant parameters (e.g. vacuum settings, filtration times) can easily be adjusted by the user.

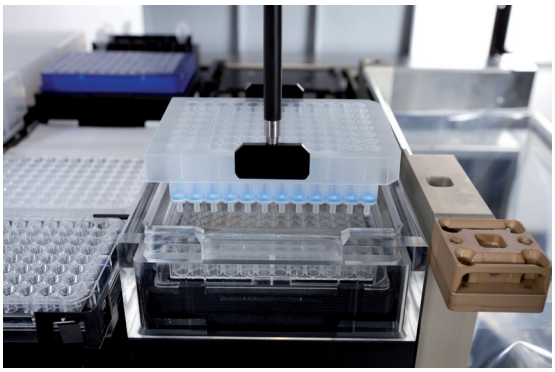

***Figure 1:*** *Transport of the NucleoSpin® RNA binding plate using the HAMILTON CO-RE Gripper.*

## Method Description

Lysed homogenized and prefiltered tissue samples are placed in Eppendorf tubes or microtiter plates on the deck. RNA extraction from cultured cells can be directly performed from a 96-well cell culture plate without prior preparation as they are lysed on the instrument. After the addition of an equal volume of binding buffer and an efficient mixing by aspiration and dispensing steps, the samples are transferred to the NucleoSpin® RNA 8-well Strips or 96-well binding plate. During the following filtration step under vacuum created by the MICROLAB® CVS, the RNA binds to the silica membrane. An optional clogg check is performed after this step. Columns are tracked and excluded from further processing if clogging is detected. Subsequently, the membrane is desalted with a wash buffer. After the DNase treatment of the samples, which is done directly on the membrane for 15min at room temperature, the silica membranes are washed three times with three different wash buffers. Once the binding plate is dry, the high purity total RNA is eluted with 50-130µl RNase-free water.

### Validation

The isolation of RNA was validated with Hela cells as well as mouse liver tissue on the Genomic STARlet. $10^5$ Hela cells and 2.5mg mouse liver tissue, respectively, were used for the isolation. Mouse liver tissue stored in RNAlater™ (Ambion) was used. Before loading to the deck, the mouse liver was manually homogenized and prefiltered. Cells were directly lysed in a 96-well cell culture plate during the process on the instrument. 8-well strips or a 96-well

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binding plate were used to isolate the RNA and the obtained yield and purity were compared with samples isolated manually. The RNA yields and purity were measured by UV spectrophometry using a Biotek Lambda Scan 200 plate reader. Total RNA quality was analyzed on an Agilent Bioanalyzer 2100 using using the RNA 6000 Nano kit.

A one step real-time qPCR was performed by using 2µl of the eluted RNA as a template. The GAPDH gene was targeted. The rt-qPCR was performed on a Light Cycler (Roche).

## Results

48 and 96 samples were processed both manually and automated on the Genomic STARlet with the 8-well strips and the 96-well binding plates, respectively. Figure 2 summarizes the obtained RNA purities and yields. The purities were between 1.9 and 2.2 for all samples (Fig. 2B and 2D). The RNA yields were between 1.34µg and 1.67µg for the cells (Fig. 2A) and between 15.36µg and 16.61ug for the mouse liver (Fig. 2C).

The quality analysis of the purified RNA on the Bioanalyzer revealed excellent quality RNA Integrity Numbers (RIN) of 9.9 to 10 for the cells and 8.0 to 8.5 for the tissues (Fig. 3). As downstream application, a Realtime qPCR analysis was performed using the purified RNA as template. Reproducible and consistant amplification results were obtained.

The Genomic STARlet could process 48 samples with the 8-well strips or 96 samples with the 96-well binding plates in less than 80min.

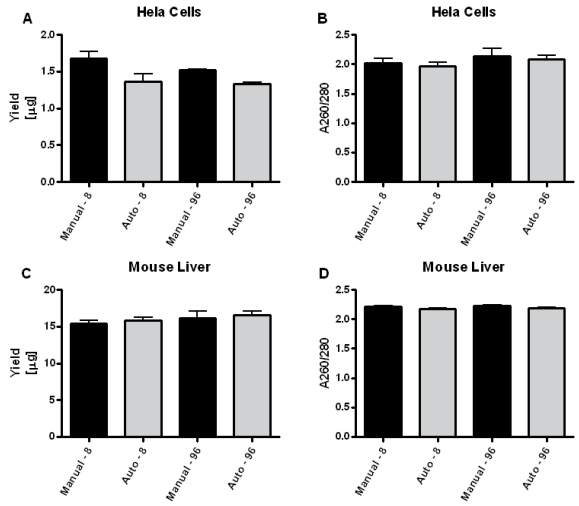


***Figure 2:*** *Shows RNA yields (A, C) and purities (B, D) after the extraction from Hela cells (A, B) and mouse liver (C, D). 8 indicates that samples were extracted with 8-well strips, 96 indicates that samples were extracted with 96-well binding plate. Standard deviations are shown.*

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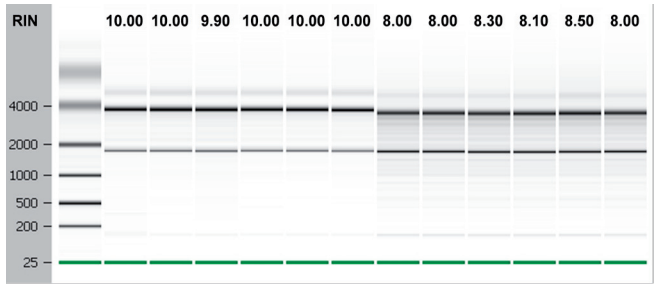


***Figure 3:*** *Gel image (Bioanalyzer 2100) from automated isolations. Samples 1-6: RNA from Hela cells; Samples 7-12: RNA from mouse liver. L: Ladder. RIN: RNA Integrity Number*

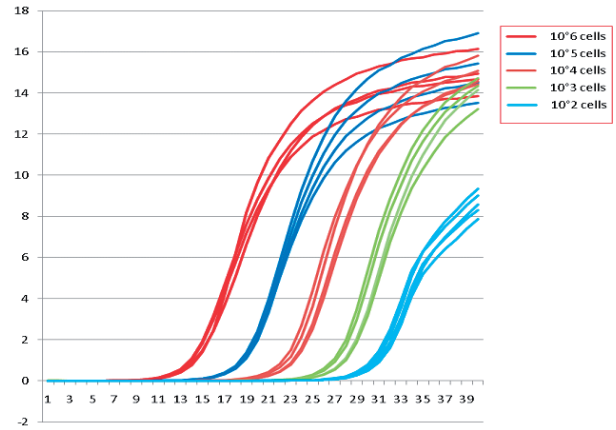


***Figure 4:*** *Real-time qPCR-analysis: RNA was isolated from a dilution series of Hela Cells (1x10⁶-1x10² cells/extraction). 2µl of the eluted RNA was used as template in a one step qRT-PCR targeting the GAPDH gene. Reproducible and consistant amplification results were obtained.*

## Discussion & Summary

HAMILTON and MACHEREY-NAGEL have designed and validated the Genomic STARlet to allow optimal reliability, throughput, yield and quality for the extraction of nucleic acids from various samples. Here, we demonstrate the automated isolation of total RNA out of Hela cells and mouse liver tissues. The automated and manual isolation resulted in similar yields and qualities of RNAs. The RNA was of excellent quality and could be used directly in further downstream applications as Realtime qPCR analysis.

Therefore, the automated isolation of RNA on the Genomic STARlet guarantees increased standardization for downstream gene expression studies.

MACHEREY-NAGEL

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Headquarters
Europe - HAMILTON AG
Via Crusch 8, CH-7402 Bonaduz
Switzerland
infoservice@hamiltonrobotics.com
Americas - HAMILTON Robotics
4970 Energy Way
Reno, Nevada 89520 USA
sales@hamiltoncompany.com

United Kingdom
HAMILTON Robotics Ltd
6120 Gnd Floor Knights Court
Solihull Parkway, Birmingham
Business Park, B37 7WY, UK
Telephone: +44-121-717-0199
Fax: +44-121-717-0209
info.gb@hamiltonrobotics.com

Germany, Austria, Benelux
HAMILTON Robotics GmbH
Fraunhoferstr. 17
D-82152 Martinsried
Germany
Telephone: +49-89-5526-49-0
Fax: +49-89-5526-49-10
info.de@hamiltonrobotics.com

France
HAMILTON Robotics S.A.R.L.
Parc du Moulin de Massy
37 rue du Saule Trapu
F-91300 Massy/France
Telephone +33-1-69-75-16-16
Fax +33-1-60-11-57-16
info.fr@hamiltonrobotics.com

Italy
HAMILTON Italia Srl
Via Paracelso 22
IT-20041Agrate Brianza (MB)
Italy
Telephone +39-39-689-33-93
Fax +39-39-605-86-25
info.it@hamiltonrobotics.com

Scandinavia
HAMILTON Robotics ApS
Sankt Annæ Plads 13, 4
DK-1250 Copenhagen K
Denmark
Telephone: +45-70-26-4499
Fax +49-89-5526-49-10
Info.de@hamiltonrobotics.com

China
HAMILTON AG Shanghai Rep. Office
Room 502, No.50 Boxia RD,
Shanghai Pudong Software Prk,
Pudong, 201203 Shanghai
Telephone +86.21.6164-6567
Fax +86.21.6106-3732
info.cn@hamiltonrobotics.com